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(54) **MHC CLASS IA OPEN CONFORMERS**

OFFENE MHC-ANPASSER DER KLASSE IA

CONFORMÈRES OUVERTES DU CMH DE CLASSE IA

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- **LUTHRA-GUPTASARMA M ET AL: "HLA-B27 lacking associated @b2-microglobulin rearranges to auto-display or cross-display residues 169-181: a novel molecular mechanism for spondyloarthropathies", FEBS LETTERS, ELSEVIER, AMSTERDAM, NL, vol. 575, no. 1-3, 24 September 2004 (2004-09-24), pages 1-8, XP004573846, ISSN: 0014-5793, DOI: 10.1016/J.FEBSLET.2004.08.037**

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Description

[0001] The present invention relates to a fusion MHC-Ia open conformer that comprises an HLA heavy chain selected from C08, A25, B58, A30, B53, and C12 covalently linked to an Fc polypeptide sequence, particularly for use in the prophylaxis or treatment of cancer, and for use as immunomodulators.

[0002] Human leukocyte antigens (HLA) belong to the classical major histocompatibility complex (MHC) protein family. The HLA complex helps the immune system distinguish the body's own proteins from proteins made by foreign invaders such as viruses and bacteria. Humans have MHC class I molecules comprising the classical (MHC-Ia) HLA-A, HLA-B, and HLA-C, and the non-classical (MHC-Ib) HLA-E, HLA-F, HLA-G and HLA-H molecules. Both categories are similar in their mechanisms of peptide binding, presentation and induced T-cell responses. The most remarkable feature of the classical MHC-Ia is their high polymorphism, while the non-classical MHC-Ib are usually non-polymorphic and tend to show a more restricted pattern of expression than their MHC-Ia counterparts.

[0003] The HLA nomenclature is given by the particular name of gene locus (e.g. HLA-**A**) followed by the allele family serological antigen (e.g. HLA-A*__02__), and allele subtypes assigned in numbers and in the order in which DNA sequences have been determined (e.g. HLA-A*02__:01__). Alleles that differ only by synonymous nucleotide substitutions (also called silent or non-coding substitutions) within the coding sequence are distinguished by the use of the third set of digits (e.g. HLA-A*02:01 :__01__). Alleles that only differ by sequence polymorphisms in the introns, or in the 5' or 3' untranslated regions that flank the exons and introns, are distinguished by the use of the fourth set of digits (e.g. HLA-A*02:01:01:__02L__) (Fig. 1).

[0004] A list of MHC-Ia alleles is provided in Table 1. For a complete list of allele subtypes visit the link: http://hla.alleles.org/alleles/class1.html.

[0005] Classical MHC-Ia molecules' principle function is to present peptides as part of the adaptive immune response. MHC-Ia molecules are trimeric structures comprising a membrane-bound heavy chain with three extracellular domains (α1, α2 and α3) that associates non-covalently with β2-microglobulin (β2m) and a small peptide which is derived from self-proteins, viruses or bacteria. The α1 and α2 domains are highly polymorphic and form a platform that gives rise to the peptide-binding groove. Juxtaposed to the conserved α3 domain is a transmembrane domain followed by an intracellular cytoplasmic tail.

[0006] To initiate an immune response classical MHC-Ia molecules present specific peptides to be recognized by TCR (T cell receptor) present on $CD8^+$ cytotoxic T lymphocytes (CTLs), while NK cell receptors present in natural killer cells (NK) recognize peptide motifs, rather than individual peptides. Under normal physiological conditions, MHC-Ia molecules exist as heterotrimeric complexes in charge of presenting peptides to CD8+ T cells and NK cells, however, MHC-Ia molecules may also be present in cells as free-heavy chains lacking β2-microglobulin and peptide, and are referred to as HLA-open conformers (Arosa et al., Trends in Immunology 2007 Mar; 28(3): 115-23) (Fig. 2). The interaction of HLA-open conformers with T cell receptors and NK cell receptors is independent of the peptide and its function is unknown.

[0007] Open conformers can be expressed at the cell surface of cells and can be detected with antibodies recognizing linear epitopes of HLA molecules without β2m and peptide (e.g. LA45, L31, HCA2 and HC-10). These antibodies have been used to detect the presence of open conformers in diverse autoimmune patients and healthy individuals (Raine et al., Rheumatology 2006;45:1338-1344). Despite their presence in patients and cell lines little is know of their mode of action. Open conformers have been mostly assessed in Ankylosing spondylitis (AS) *+HLA-B27* patients, where HLA-B27 open conformers have been hypothesized to induce autoimmunity, their function in other autoimmune patients has not been yet addressed.

[0008] Ciprandi et al. (Allergy 2008, 63, 1335-1338) discuss the association of soluble HLA molecules with allergic rhinitis. Topalian et al. (Cancer Cell 27 (2015), 450-461) review checkpoint blockade in cancer treatment. WO9958557 discloses HLA-B27 dimers in diagnosis of spondyloarthropathy.

[0009] Here the inventors disclose for the first time that the classical MHC-Ia (HLA-A, HLA-B and HLA-C) family of molecules when present as open conformers (heavy chains without β2m) are useful therapeutics for their immunomodulatory properties and use in the treatment of cancer.

[0010] Overall, modulating the immune contexture of tumors favoring the infiltration of M1 type macrophages, cytotoxic CD8 T-cells, and Th1 cells, and/or reducing the infiltration of MDSCs and M2 type macrophages is a promising therapeutic avenue to treat cancer that is explored here with the use of HLA open conformers proteins in diverse cancer indications.

Terms and definitions

[0011] Amino acid sequences are given from amino to carboxyl terminus. Capital letters for sequence positions refer to L-amino acids in the one-letter code (Stryer, Biochemistry, 3rd ed. p. 21).

[0012] The term *open conformer* as used in the present specification refers to an isolated HLA heavy chain molecule not associated to β2-microglobulin either as a monomer or as a dimer (homodimer or heterodimer). Certain embodiments of the open conformers disclosed herein are fusion protein monomers or dimers, wherein the HLA heavy chain is covalently linked to a stabilizing polypeptide region, particularly a crystallizable fragment immunoglobulin domain.

[0013] In the context of the present specification the terms *sequence identity* and *percentage of sequence identity* refer to the values determined by comparing two aligned sequences. Methods for alignment of sequences for comparison are well-known in the art. Alignment of sequences for comparison may be conducted by the local homology algorithm of Smith and Waterman, Adv. Appl. Math. 2:482 (1981), by the global alignment algorithm of Needleman and Wunsch, J. Mol. Biol. 48:443 (1970), by the search for similarity method of Pearson and Lipman, Proc. Nat. Acad. Sci. 85:2444 (1988) or by computerized implementations of these algorithms, including, but not limited to: CLUSTAL, GAP, BESTFIT, BLAST, FASTA and TFASTA. Software for performing BLAST analyses is publicly available, e.g., through the National Center for Biotechnology-Information (http://blast.ncbi.nlm.nih.gov/). One example for comparison of amino acid sequences is the BLASTP algorithm that uses the default settings: Expect threshold: 10; Word size: 3; Max matches in a query range: 0; Matrix: BLOSUM62; Gap Costs: Existence 11, Extension 1; Compositional adjustments: Conditional compositional score matrix adjustment. One such example for comparison of nucleic acid sequences is the BLASTN algorithm that uses the default settings: Expect threshold: 10; Word size: 28; Max matches in a query range: 0; Match/Mismatch Scores: 1.-2; Gap costs: Linear. Unless otherwise stated, sequence identity values provided herein refer to the value obtained with the BLAST suite of programs (Altschul et al., J. Mol. Biol. 215:403-410 (1990)) using the above identified default parameters for protein and nucleic acid comparison, respectively.

[0014] In the context of the present specification, the term *major histocompatibility complex* (MHC) is used in its meaning known in the art of cell biology and immunology; it refers to a cell surface molecule that displays a specific fraction (peptide), also referred to as an epitope, of a protein. There a two major classes of MHC molecules: class **I** and class **II.** Within the MHC class I two groups can be distinguished based on their polymorphism: a) the classical (MHC-Ia) with corresponding polymorphic HLA-A, HLA-B, and HLA-C genes, and b) the non-classical (MHC-Ib) with corresponding less polymorphic HLA-E, HLA-F, HLA-G and HLA-H genes.

[0015] MHC class **I** heavy chain molecules usually (i.e. when not in open conformer form) occur as an alpha chain linked to a unit of the non-MHC molecule β2-microglobulin. The alpha chain comprises, in direction from the N-terminus to the C-terminus, a signal peptide, three extracellular domains (α1-3, with α1 being at the N terminus), a transmembrane region and a C-terminal cytoplasmic tail. The peptide being displayed or presented is held by the peptide-binding groove, in the central region of the α1/α2 domains.

[0016] In the context of the present specification, the term *β2-microglobulin* domain is used in its meaning known in the art of cell biology and biochemistry; it refers to a non-MHC molecule that is part of the MHC class I heterodimer molecule. In other words, it constitutes the β chain of the MHC class **I** heterodimer.

[0017] In the context of the present specification, the term *human leukocyte antigen (HLA)* is used in its meaning known in the art of cell biology and biochemistry; it refers to gene loci encoding the human MHC class **I** proteins. The three major classical MHC-Ia genes are HLA-A, HLA-B and HLA-C, and all of these genes have a varying number of alleles (Table 1). Closely related alleles are combined in subgroups of a certain allele. For example the allele HLA-B57 has more than 100 closely related alleles that vary in one or more amino acids, according to the WHO Nomenclature Committee for Factors of the HLA System, labelled HLA-B*57:01:01 to HLA-B*57:82. The full or partial sequence of all known HLA genes and their respective alleles are available to the person skilled in the art in specialist databases such as IMGT/HLA (http://www.ebi.ac.uk/ipd/imgt/hla/).

[0018] In the context of the present specification, the term *antibody* is used in its meaning known in the art of cell biology and immunology; it refers to whole antibodies including but not limited to immunoglobulin type G (IgG), type A (IgA), type D (IgD), type E (IgE) or type M (IgM), any antigen binding fragment or single chains thereof and related or derived constructs. A whole antibody is a glycoprotein comprising at least two heavy (H) chains and two light (L) chains inter-connected by disulfide bonds. Each heavy chain is comprised of a heavy chain variable region (V_H) and a heavy chain constant region (C_H). The heavy chain constant region is comprised of three domains, C_H1, C_H2 and C_H3. Each light chain is comprised of a light chain variable region (abbreviated herein as V_L) and a light chain constant region (C_L). The light chain constant region is comprised of one domain, C_L. The variable regions of the heavy and light chains contain a binding domain that interacts with an antigen. The constant regions of the antibodies may mediate the binding of the immunoglobulin to host tissues or factors, including various cells of the immune system (e.g., effector cells) and the first component of the classical complement system.

[0019] The term *antibody-like molecule* in the context of the present specification refers to a molecule capable of specific binding to another molecule or target with high affinity / a Kd ≤ 10E-8 mol/l. An antibody-like molecule binds to its target similarly to the specific binding of an antibody. The term *antibody-like molecule* encompasses a repeat protein, such as a designed ankyrin repeat protein (Molecular Partners, Zürich), a polypeptide derived from armadillo repeat proteins, a polypeptide derived from leucine-rich repeat proteins and a polypeptide derived from tetratricopeptide repeat proteins.

[0020] In the context of the present specification, the term *crystallizable fragment (Fc) region* is used in its meaning known in the art of cell biology and immunology; it refers to a fraction of an antibody comprising two identical heavy chain fragments comprised of a C_H2 and a C_H3 domain, covalently linked by disulfide bonds.

[0021] In the context of the present specification, the term *dimer* refers to a unit consisting of two subunits.

[0022] In the context of the present specification, the term *homodimer* refers to a dimer comprised of two subunits that are either identical or are highly similar members of the same class of subunits. One example for a homodimer would be a dimer consisting of two subunits independently selected from the list of HLA alleles. In certain embodiments, homodimers consist of two identical HLA alleles.

[0023] In the context of the present specification, the term *amino acid linker* refers to a polypeptide of variable length that is used to connect two polypeptides in order to generate a single chain polypeptide. Exemplary embodiments of linkers useful for practicing the invention specified herein are oligopeptide chains consisting of 1, 2, 3, 4, 5, 10, 20, 30, 40 or 50 amino acids. A non-limiting example of an amino acid linker is the polypeptide GGGGSGGGGS (SEQ ID No. 001) that links an HLA-heavy chain polypeptide with an Fc domain.

[0024] In the context of the present specification, the term *checkpoint inhibitory agent* or *checkpoint inhibitory antibody* is meant to encompass an agent, particularly a (non-agonist) antibody (or antibody-like molecule) capable of disrupting the signal cascade leading to T cell inhibition after T cell activation as part of what is known in the art the immune checkpoint mechanism. Non-limiting examples of a *checkpoint inhibitory agent* or *checkpoint inhibitory antibody* include antibodies to CTLA-4 (Uniprot P16410), PD-1 (Uniprot Q15116), PD-L1 (Uniprot Q9NZQ7), B7H3 (CD276; Uniprot Q5ZPR3), Tim-3, Gal9, VISTA, or Lag3.

[0025] In the context of the present specification, the term *checkpoint agonist agent* or *checkpoint agonist antibody* is meant to encompass an agent, particularly but not limited to an antibody (or antibody-like molecule) capable of engaging the signal cascade leading to T cell activation as part of what is known in the art the immune checkpoint mechanism. Non-limiting examples of receptors known to stimulate T cell activation include CD122 and CD137 (4-1BB; Uniprot 007011). The term *checkpoint agonist agent* or *checkpoint agonist antibody* encompasses agonist antibodies to CD137 (4-1BB), CD134 (OX40), CD357 (GITR), CD278 (ICOS), CD27, CD28.

[0026] In the context of the present specification, the term (immune) checkpoint modulatory agent encompasses *checkpoint inhibitory agents, checkpoint inhibitory antibodies, checkpoint agonist agents* and *checkpoint agonist antibodies.*

Specific description of the invention

[0027] The present invention relates to a fusion MHC-Ia open conformer, wherein said fusion MHC-Ia open conformer comprises or essentially consists of a first monomer or a first and a second monomer, wherein

a. said first monomer, or each of said first and second monomer independently of the other monomer, comprises an HLA heavy chain selected from C08, A25, B58, A30, B53, and C12, and

b. wherein said first monomer, or each of said first and second monomer are covalently linked to an Fc polypeptide sequence,

wherein the HLA-heavy chain only consists of the HLA alpha 1, 2 and 3 domains.

[0028] In certain embodiments, the fusion MHC-Ia open conformer comprises a first monomer or a first and a second monomer, and each monomer independently of the other monomer comprises a HLA heavy chain.

[0029] According to a second aspect of the invention a fusion MHC-Ia open conformer is provided:

- for use as a medicament,
- particularly for use in the treatment or prevention of cancer, or
- particularly for use as an immunomodulatory agent,
    - particularly in a treatment of an infectious disease,
        - more particularly for use in prevention, treatment or therapy of human immunodeficiency virus (HIV), hepatitis A, B, C, virus (HAV HBV, HCV respectively), influenza virus, Respiratory Syncytial Virus (RSV), measles virus, herpes viruses and/or yellow fever virus.

[0030] In certain embodiments of the second aspect of the invention or of any above-mentioned alternative to the second aspect of the invention, the cancer is colon cancer or pancreatic cancer.

[0031] n aspect of the invention relates to a fusion MHC-Ia open conformer. The fusion MHC-Ia open conformer comprises, or essentially consists of, a first HLA heavy chain monomer or a first and a second HLA heavy chain monomer. Each of these HLA heavy chain monomer independently of the other comprises or essentially consists of a HLA heavy chain selected from C08, A25, B58, A30, B53, and C12. The fusion MHC open conformer additionally comprises an Fc polypeptide sequence. In certain embodiments, the HLA monomer sequence is situated at the N terminus of the fusion

MHC open conformer, and the Fc construct is located towards the C terminus. In certain embodiments, an amino acid linker joins the HLA-heavy chain and the Fc fragment.

[0032] The fusion MHC-Ia open conformer additionally comprises a polypeptide domain known to metabolically stabilize a polypeptide *in vivo.* One example of such a stabilizing domain is an Fc (crystallisable fragment) domain of an immunoglobulin, particularly the Fc polypeptide domain of a gamma immunoglobulin. The HLA-heavy chain and the stabilizing domain may optionally be joined by an amino acid linker. An open conformer fusion protein comprising the HLA chain and an immunoglobulin Fc fragment is henceforth termed HLA-Fc open conformer or HLA_2-Fc herein.

[0033] The presence of the Fc domain in the fusion protein facilitates increasing the solubility, stability, avidity, half-life, and from a technological point of view, cost-effective production and purification in mammalian systems (protein A or G purification).

[0034] In certain embodiments of any one of the aspects of the invention, the isolated MHC-Ia open conformer or fusion MHC-Ia open conformer consists of two subunits independently selected from the above HLA-alleles. In certain embodiments, homodimers consist of two identical HLA-alleles.

[0035] In certain embodiments of any one of the aspects of the invention, the isolated MHC-Ia open conformer or fusion MHC-Ia open conformer comprise two identical HLA polypeptide chains. In certain embodiments, the isolated MHC-Ia open conformer or fusion MHC-Ia open conformer comprises two different HLA polypeptide chains.

[0036] In certain embodiments of any one of the aspects of the invention, the isolated MHC-Ia open conformer or fusion MHC-Ia open conformer additionally comprises a peptide epitope fragment.

[0037] In certain embodiments of any one of the aspects of the invention, a peptide epitope fragment is non-covalently attached to the polypeptide within the antigen presenting domain of the HLA peptide chain.

[0038] In certain embodiments of any one of the aspects of the invention, the first and/or second monomer additionally comprises a peptide epitope fragment.

[0039] The fusion MHC-Ia open conformer comprises only the extracellular HLA-alpha 1, HLA-alpha 2 and HLA-alpha 3 domains. In these embodiments, the transmembrane and intracellular domains of the HLA heavy chains are not included in the therapeutic polypeptide of the invention in order to allow its extracellular expression in recombinant cells. The person skilled in the art can easily identify the respective domains even in previously unknown HLA-sequences by pair-wise sequence alignment with annotated HLA-sequences.

[0040] In certain embodiments of any one of the aspects of the invention, the fusion MHC-Ia open conformers comprise an Fc domain. In certain particular embodiments, the Fc domain comprises heavy chain constant regions C_H2 and C_H3 from immunoglobulin type G (IgG), type A (IgA), type D (IgD), type E (IgE) or type M (IgM).

[0041] In certain embodiments of any one of the aspects of the invention, the fusion MHC-Ia open conformers comprise an amino acid linker joining a stabilizing domain, particularly an Fc domain, to the HLA polypeptide. In certain particular embodiments, the amino acid linker comprises 1 to 50 amino acids, particularly 5 to 40 amino acids, more particularly 10 to 30 amino acids, even more particularly 15 to 25 amino acids that link the HLA-heavy chain to the Fc domain as one single polypeptide chain.

[0042] In certain embodiments of any one of the aspects of the invention, the isolated MHC-Ia open conformers or fusion MHC-Ia open conformers, are provided as parenteral dosage form, particularly confectioned for injection. In certain embodiments, the immune checkpoint inhibitor agent or agonist agent is provided as parenteral dosage form, particularly confectioned for injection. In certain embodiments, both the MHC-Ia open conformers and the immune checkpoint inhibitor agent or agonist agent are present in the same administration form.

[0043] In certain embodiments of the third aspect of the invention, the fusion MHC-Ia open conformer is for use as a medicament.

[0044] In certain embodiments of the third aspect of the invention, the fusion MHC-Ia open conformer is for use in the treatment or prevention of cancer, in particular for colon cancer or pancreatic cancer.

[0045] In certain embodiments of the third aspect of the invention, the fusion MHC-Ia open conformer is for use as an immunomodulatory agent, particularly for use as negative modulator of regulatory T cells (Treg). In certain embodiments, the fusion MHC-Ia open conformer is for use in the treatment of infectious diseases. In certain embodiments, the fusion MHC-Ia open conformer is for use in the treatment of human immunodeficiency virus (HIV) infection, hepatitis A, hepatitis B, hepatitis C, influenza, respiratory syncytial virus (RSV) infection, measles, herpes and yellow fever.

[0046] According to a fourth aspect of the invention, a nucleic acid molecule encoding an Fc open conformer monomer, according to the above aspects of the invention is provided for use in the treatment or the therapy of cancer or for use as an immunomodulatory agent, particularly in a treatment of an infectious disease. Expression of the open conformer *in vivo* from the nucleic acid molecule will, after dimerization, lead to the fusion protein polypeptide of the invention. The concept of expressing pharmaceutically active polypeptides from nucleic acids encoding them in the patient's body is well known and may confer significant benefits to the patient.

[0047] In certain embodiments of the fourth aspect of the invention or any above-mentioned alternative thereof, the cancer is colon cancer or pancreatic cancer.

[0048] In certain embodiments, the nucleic acid molecule encodes MHC-Ia open conformers monomers, particularly

an Fc open conformer monomer that comprises an amino acid linker and/or an Fc (fragment crystallizable) domain, and is used in the treatment or the therapy of cancer, in particular colon or pancreatic cancer.

[0049] According to an alternative aspect of the invention a recombinant expression vector comprising the nucleic acid molecule according to the fourth aspect of the invention (and its alternative aspects) is provided for use in the treatment or the therapy of cancer, in particular colon or pancreatic cancer.

[0050] In certain embodiments the recombinant expression vector is a plasmid comprising a promoter that is operable in a mammalian cell, particularly in a human cell. The promoter is operably linked to the nucleic acid molecule of the invention.

[0051] According to a fifth aspect of the invention a virus comprising the nucleic acid molecule according to the fourth aspect of the invention (and its alternative aspects) is provided for use in the treatment or the therapy of cancer, in particular colon or pancreatic cancer, or for use as an immunomodulatory agent, particularly in a treatment of an infectious disease. The nucleic acid molecule is under control of a promoter sequence operable in a mammalian cell, particularly in a human cell. In certain embodiments, the virus is an adenovirus, adeno-associated virus, a herpes virus or a lentivirus.

[0052] According to a sixth aspect of the invention an *in vitro* genetically modified host cell comprising the nucleic acid molecule according to the fourth aspect of the invention (and its alternative aspects) is provided.

[0053] According to a seventh aspect of the invention, a combination medicament is provided, wherein the combination medicament comprises:

- isolated MHC-Ia open conformers or fusion MHC-Ia open conformers, according to any one of the above aspects or embodiments of the invention, and
- an immune checkpoint modulatory agent selected from

    ◦ an immune checkpoint inhibitor agent (CPI) selected from:

    - an inhibitor of cytotoxic T-lymphocyte-associated protein 4 (CTLA4; also known as CD152) interaction with either B7-1 (CD80) and/or B7-2 (CD86), particularly a polypeptide ligand to CTLA-4 or to cd80 or to cd86 such as for example an antibody,
    - an inhibitor of the interaction of programmed cell death protein 1 (PD-1; also known as CD279) with its ligand PD-L1 (also known as CD274; UniProt ID: Q9NZQ7) and/or PD-L2 (also known as CD273; Uni Prot ID: Q9BQ51), particularly a polypeptide ligand to PD-1 or to PD-L1 or to PD-L2 such as for example an antibody, and
    - an inhibitory polypeptide ligand, particularly an antibody, of T cell immunoglobulin and mucin domain-containing 3 (TIM-3), and

    ◦ a checkpoint agonist agent, particularly a checkpoint agonist antibody selected to bind to and activate the tumor necrosis factor receptor 4-1BB (also known as CD137 or TNFRSF9).

[0054] In certain embodiments, the immune checkpoint inhibitor agent is an inhibitor of interaction of CTLA4 with CD80 or CD86.

[0055] In certain embodiments, the immune checkpoint inhibitor agent is ipilimumab (Yervoy; CAS No. 477202-00-9).

[0056] In certain embodiments, the immune checkpoint inhibitor agent is an inhibitor of interaction of programmed cell death protein 1 (PD-1) with its receptor PD-L1. In certain embodiments, the immune checkpoint inhibitor agent is selected from the clinically available antibody drugs nivolumab (Bristol-Myers Squibb; CAS No 946414-94-4), pembrolizumab (Merck Inc.; CAS No. 1374853-91-4), pidilizumab (CAS No. 1036730-42-3), atezolizumab (Roche AG; CAS No. 1380723-44-3), and Avelumab (Merck KGaA; CAS No. 1537032-82-8).

[0057] In certain embodiments, the immune checkpoint agonist agent is utomilumab (PF-05082566), a fully human IgG2 monoclonal antibody against 4-1BB currently undergoing clinical trials.

[0058] In certain embodiments, the checkpoint modulatory agent is a polypeptide selected from an antibody, an antibody fragment, and an antibody-like molecule, and the polypeptide is selectively reactive to a checkpoint mediator. In certain embodiments, the checkpoint mediator is selected from CTLA4, PD-1, CD80, CD86, PD-L1, and PD-L2, TIM-3, 4-1BB and 4-1BBL.

Brief description of the figures

[0059]

Fig. 1 shows the nomenclature of MHC class I molecules.

Fig. 2 shows the schematic representation of HLA-heterotrimers and HLA-open conformers (free-heavy chains). Both forms may exist at the cell surface of antigen presenting cells (APC cells). The inventors propose that the interaction of open conformers with immunoregulatory receptors (KIR's, LIL's, PTPRJ, etc.) is different in affinity and thus modified to induce immune responses that favour anti-tumor immunity.

Fig. 3 shows the schematic representation of HLA-Fc and β2m DNA cassettes and expression of HLA-β2m-Fc molecules from CHO cells. A) alpha 1, 2 and 3 domains of MHC-la heavy chains (HLA-heavy chain) are inserted into a human IgG4-Fc vector cassette; and the human- β2microglobulin inserted in a separate vector cassette. B) Transfections in Chinese hamster ovary cells (CHO) cells are performed using both the HLA-Fc-vector + β2m-vector at a ratio of 1:1 for the extracellular production of the HLA-β2m-Fc protein. Supernatants were collected and HLA-β2m-Fc purified using standard antibody purification protocols. β2m is removed from the HLA-β2m-Fc complex and following HLA-Fc monomers are refolded to form HLA_2-Fc homodimers.

Fig. 4 shows the separation of β2m from the HLA-β2m-Fc complex and purification and refolding of HLA_2-Fc by SEC. A) Chromatography histogram plot of HLA-β2m-Fc molecules in Urea-Tris-BME denaturing buffer show the dissociation of HLA-Fc-free heavy chains from β2m using Sephacryl S-100 HR columns by SEC. B) and C) SDS-page gels stained with coomassie blue show the presence of β2m before and after SEC. B) shows HLA-B2m-Fc molecules before being separated in SEC, and C) show HLA_2-Fc molecules recovered and re-folded following SEC.

Fig. 5 shows the interaction HLA_2-Fc ($A25_2$-Fc, $A30_2$-Fc, $B27_2$-Fc, $B53_2$-Fc, $B57_2$-Fc, $B58_2$-Fc, $C08_2$-Fc and $C12_2$-Fc) to different immune regulatory receptors of leukocytes populations by enzyme-linked immunosorbent assay (ELISA). A) hu KIR3DL1, B) hu KIR3DL2, and C) hu KIR3DL3 are expressed in NK cells and subpopulations of T cells. D) LILRB1, and E) LILRB2 expressed mostly in myeloid cells, F) PirB (murine homologue to LILRB) and G) PTPRJ (on leukocytes is preferentially expressed in MDSCs cells and activated T cells)

Fig. 6 shows that HLA_2-Fc molecules ($A25_2$-Fc, $A30_2$-Fc, $B27_2$-Fc, $B53_2$-Fc, $B57_2$-Fc, $B58_2$-Fc, $C08_2$-Fc, and $C12_2$-Fc) invariably block mouse $CD4^+$ T cell conversion into iTreg. Incubation of HLA_2-Fc in a dose dependent manner with naive $CD4^+$ T cells blocks the conversion to iTregs. A-B) HLA_2-Fc molecules blocks the expression of FoxP3 (differentiation marker of Tregs) in optimal culture conditions for iTreg differentiation (10μg/mL) Control HLA-β2m-Fc molecules, isotype, media supplemented with TGFβ and IL-2 and media w/o supplementation demonstrate the specific influence of HLA_2-Fc on iTreg conversion.

Fig. 7 shows that HLA_2-Fc ($A25_2$-Fc, $A30_2$-Fc, $B27_2$-Fc, $B53_2$-Fc, $B57_2$-Fc, $B58_2$-Fc, $C08_2$-Fc and $C12_2$-Fc) suppresses lymphoma T cells. A-E) suppression assays to determine the proliferation of cells in the presence of HLA_2-Fc molecules or control HLA-β2m-Fc molecules. HLA_2-Fc suppress human (Jurkat) and mouse (EG.7) lymphoma cell lines in a dose dependent manner (μg/200μL), other cell lines such as Daudi, B cell lymphoma; SK-N-AS, neuroblastoma; and L540, human Hodgkin lymphoma were assessed but not suppression was observed from HLA_2-Fc molecules in optimal culture conditions. Other cell lines such as L428, human Hodgkin lymphoma; L1236, human Hodgkin lymphoma; IMR-5, neuroblastoma; and M130428, Melanoma were also tested but no suppression was observed.

Fig. 8 shows that HLA_2-Fc ($A30_2$-Fc, $B58_2$-Fc, and $C08_2$-Fc) as monotherapy or in combination with PD-1 antibodies can reduce the size of tumors in the C38 murine syngeneic colon carcinoma model. A) Experimental design of injection time points of colon carcinoma cells (C38) and injection of compounds. B) Mean average tumor volume mm^3 of $A30_2$-Fc treated groups (n=5). C) Mean average tumor volume mm^3 of $B58_2$-Fc treated groups (n=5). D) Mean average tumor volume mm^3 of $C08_2$-Fc treated groups (n=5). The experimental design of injection time points of cells and injection of substances was as follow: vehicle PBS Q3Dx6, isotype (10mg/Kg) Q3Dx6; HLA_2-Fc (10 mg/Kg) Q3Dx6; PD-1 biwk × 2 (200 μg); and HLA_2-Fc + PD-1 (Q3Dx6 & biwk × 2, respectively). Tumor volumes are expressed as mean ± SEM and analysed by two-way ANOVA followed by Bonferroni post-hoc analysis, *p<0.05, **p<0.01, n.s.= not significant. Q= days between injections; Dx= number of injections; biwk= twice a week.

Fig. 9 shows that HLA_2-Fc ($B27_2$-Fc and $B57_2$-Fc) in combination with CTLA4 or PD-1 antibodies reduce the size of tumors in the MC38-OVA or C38 murine syngeneic colon carcinoma model. A) Mean average tumor volume mm^3 of $B27_2$-Fc treated groups (n=6). B) Mean average tumor volume mm^3 of $B57_2$-Fc treated groups (n=6). The experimental design of injection time points of cells and injection of substances was as follow: vehicle PBS Q3Dx6, isotype (10mg/Kg) Q3Dx6; HLA_2-Fc (10 mg/Kg) Q3Dx6; CTLA-4 Q3Dx2 (d1= 100 μg; d4 = 50

μg), PD-1 biwk × 2 (200 μg); HLA_2-Fc + CTLA-4 (Q3Dx6 & Q3Dx2, respectively), and HLA_2-Fc + PD-1 (Q3Dx6 & biwk × 2, respectively). Tumor volumes are expressed as mean ± SEM and analysed by two-way ANOVA followed by Bonferroni post-hoc analysis, **p<0.01, ***p<0.001, n.s.= not significant. Q= days between injections; Dx= number of injections; biwk= twice a week.

Fig. 10 shows the in vivo study of $A25_2$-Fc in combination with PD-1 and 4-1BB antibodies in large tumors of the pancreatic Pan02 syngeneic mouse model. A) Mean average tumor volume in mm^3 of $A25_2$-Fc treated animals (n=6). B) % Δtumor inhibition of treated mice groups compared to control. The experimental design of injection time points of substances was as follow: isotype (5 mg/Kg) biwk × 2; $A25_2$-Fc (5 mg/Kg) biwk × 2; 4-1BB antibody (1 mg/Kg) biwk × 2 injections; $A25_2$-Fc + 4-1BB (5 mg/Kg and 1 mg/Kg, respectively) biwk × 2; PD-1 antibody (5 mg/Kg) biwk × 2; and $A25_2$-Fc + PD-1 (5 mg/Kg each) biwk × 2. Tumor volumes are expressed as mean ± SEM and analysed by two-way ANOVA followed by Bonferroni post-hoc analysis *p<0.05. Δtumor inhibition is calculated from the ΔT/ΔC tumor growth ratio, which represents the growth of the tumor in % from the beginning of the treatment (e.g. 300 mm^3), to the end volume of the treatment (e.g. 1000 mm^3) compared to isotype. biwk= twice a week.

Fig. 11 shows the immune contexture of Tumor Infiltrating Lymphocytes (TILs) analysis from Pan02 pancreatic cancer mice with large tumors treated with $A25_2$-Fc, 4-1BB and PD-1 by flow cytometry (continuation of experiment in Fig. 10). Relevant leukocytes analysed infiltrating the tumor: A) CD3+ T cells, CD4+ T cells, Regulatory T cells (Treg), CD8+ T cells, and the CD8+/Treg ratio. B) Granulocytes, Macrophages, Macrophage M1-type, Macrophage M2-type, and Myeloid Derived Suppressor Cells (MDSCs). C) M1/M2 macrophage ratio, Monocytes, Natural killer cells (NK), and Natural KillerT cells (NKT). Leukocytes % are expressed as box plots showing sample maxima and minima, and each group analysed by one-way ANOVA followed by Dunnet post-hoc analysis *p<0.05; **p<0.01; ***p<0.001.

Fig. 12 shows the immune contexture of blood leukocyte analysis from Pan02 pancreatic cancer mice with large tumors treated with $A25_2$-Fc, 4-1BB and PD-1 by flow cytometry (continuation of experiment in Fig. 10). Relevant leukocytes analysed present in the blood: A) CD3+ T cells, CD4+ T cells, Regulatory T cells (Treg), CD8+ T cells, and CD8+/Treg ratio. B) Th1 cells (CD4+ T cells IFNy+), Natural Killer cells (NK), and Natural Killer T cells (NKT). C) Monocytes, Granulocyte-Myeloid Derived suppressor cells (G-MDSCs), and Monocytic-Myeloid Derived Suppressor cells (M-MDSCs). Leukocytes % are expressed as box plots showing sample maxima and minima, and each group analysed by one-way ANOVA followed by Dunnet post-hoc analysis *p<0.05; **p<0.01; ***p<0.001.

Fig. 13 shows the in vivo study of $A30_2$-Fc in combination with PD-1 and 4-1BB antibodies in large tumors of the pancreatic Pan02 syngeneic mouse model. A) Mean average tumor volume in mm^3 of $A30_2$-Fc treated animals (n=6). B) % Δtumor inhibition of treated mice groups compared to control. The experimental design of injection time points of substances was as follow: isotype (5 mg/Kg) biwk × 2; $A30_2$-Fc (5 mg/Kg) biwk × 2; 4-1BB antibody (1 mg/Kg) biwk × 2 injections; $A30_2$-Fc + 4-1BB (5 mg/Kg and 1 mg/Kg, respectively) biwk × 2; PD-1 antibody (5 mg/Kg) biwk × 2; and $A30_2$-Fc + PD-1 (5 mg/Kg each) biwk × 2. Tumor volumes are expressed as mean ± SEM and analysed by two-way ANOVA followed by Bonferroni post-hoc analysis. Δtumor inhibition is calculated from the ΔT/ΔC tumor growth ratio, which represents the growth of the tumor in % from the beginning of the treatment (e.g. 300 mm^3), to the end volume of the treatment (e.g. 1000 mm^3) compared to isotype. biwk= twice a week.

Fig. 14 shows the immune contexture of Tumor Infiltrating Lymphocytes (TILs) analysis from Pan02 pancreatic cancer mice with large tumors treated with $A30_2$-Fc, 4-1BB and PD-1 by flow cytometry (continuation of experiment in Fig. 13). Relevant leukocytes analysed infiltrating the tumor: A) CD3+ T cells, CD4+ T cells, Regulatory T cells (Treg), CD8+ T cells, and the CD8+/Treg ratio. B) Granulocytes, Macrophages, Macrophage M1-type, Macrophage M2-type, and Myeloid Derived Suppressor Cells (MDSCs). C) M1/M2 macrophage ratio, Monocytes, Natural killer cells (NK), and Natural KillerT cells (NKT). Leukocytes % are expressed as box plots showing sample maxima and minima, and each group analysed by one-way ANOVA followed by Dunnet post-hoc analysis *p<0.05; **p<0.01; ***p<0.001; ****p<0.0001.

Fig. 15 shows the immune contexture of blood leukocyte analysis from treated Pan02 pancreatic cancer mice with large tumors treated with $A30_2$-Fc, 4-1BB and PD-1 by flow cytometry (continuation of experiment in Fig. 13). Relevant leukocytes analysed present in the blood: A) CD3+ T cells, CD4+ T cells, Regulatory T cells (Treg), CD8+ T cells, and CD8+/Treg ratio. B) Th1 cells (CD4+ T cells IFNy+), Natural Killer cells (NK), and Natural

Killer T cells (NKT). C) Monocytes, Granulocyte-Myeloid Derived suppressor cells (G-MDSCs), and Monocytic-Myeloid Derived Suppressor cells (M-MDSCs). Leukocytes % are expressed as box plots showing sample maxima and minima, and each group analysed by one-way ANOVA followed by Dunnet post-hoc analysis *p<0.05; **p<0.01; ***p<0.001; ****p<0.0001.

Fig. 16 shows the in vivo study of $B27_2$-Fc in combination with PD-1 and 4-1BB antibodies in large tumors of the pancreatic Pan02 syngeneic mouse model. A) Mean average tumor volume in mm^3 of $B27_2$-Fc treated animals (n=6). B) % Δtumor inhibition of treated mice groups compared to control. The experimental design of injection time points of substances was as follow: isotype (5 mg/Kg) biwk × 2; $B27_2$-Fc (5 mg/Kg) biwk × 2; 4-1BB antibody (1 mg/Kg) biwk × 2 injections; $B27_2$-Fc + 4-1BB (5 mg/Kg and 1 mg/Kg, respectively) biwk × 2; PD-1 antibody (5 mg/Kg) biwk × 2; and $B27_2$-Fc + PD-1 (5 mg/Kg each) biwk × 2. Tumor volumes are expressed as mean ± SEM and analysed by two-way ANOVA followed by Bonferroni post-hoc analysis. Δtumor inhibition is calculated from the ΔT/ΔC tumor growth ratio, which represents the growth of the tumor in % from the beginning of the treatment (e.g. 300 mm^3), to the end volume of the treatment (e.g. 1000 mm^3) compared to isotype. biwk= twice a week.

Fig. 17 shows the immune contexture of Tumor Infiltrating Lymphocytes (TILs) analysis from Pan02 pancreatic cancer mice with large tumors treated with $B27_2$-Fc, 4-1BB and PD-1 by flow cytometry (continuation of experiment in Fig. 16). Relevant leukocytes analysed infiltrating the tumor: A) CD3+ T cells, CD4+ T cells, Regulatory T cells (Treg), CD8+ T cells, and the CD8+/Treg ratio. B) Granulocytes, Macrophages, Macrophage M1-type, Macrophage M2-type, and Myeloid Derived Suppressor Cells (MDSCs). C) M1/M2 macrophage ratio, Monocytes, Natural killer cells (NK), and Natural KillerT cells (NKT). Leukocytes % are expressed as box plots showing sample maxima and minima, and each group analysed by one-way ANOVA followed by Dunnet post-hoc analysis *p<0.05; **p<0.01; ***p<0.001; ****p<0.0001.

Fig. 18 shows the immune contexture of blood leukocyte analysis from treated Pan02 pancreatic cancer mice with large tumors treated with $B27_2$-Fc, 4-1BB and PD-1 by flow cytometry (continuation of experiment in Fig. 16). Relevant leukocytes analysed present in the blood: A) CD3+ T cells, CD4+ T cells, Regulatory T cells (Treg), CD8+ T cells, and CD8+/Treg ratio. B) Th1 cells (CD4+ T cells IFNy+), Natural Killer cells (NK), and Natural Killer T cells (NKT). C) Monocytes, Granulocyte-Myeloid Derived suppressor cells (G-MDSCs), and Monocytic-Myeloid Derived Suppressor cells (M-MDSCs). Leukocytes % are expressed as box plots showing sample maxima and minima, and each group analysed by one-way ANOVA followed by Dunnet post-hoc analysis *p<0.05; **p<0.01; ***p<0.001; ****p<0.0001.

Fig. 19 shows the in vivo study of $B53_2$-Fc in combination with PD-1 and 4-1BB antibodies in large tumors of the pancreatic Pan02 syngeneic mouse model. A) Mean average tumor volume in mm^3 of $B53_2$-Fc treated animals (n=6). B) % Δtumor inhibition of treated mice groups compared to control. The experimental design of injection time points of substances was as follow: isotype (5 mg/Kg) biwk × 2; $B53_2$-Fc (5 mg/Kg) biwk × 2; 4-1BB antibody (1 mg/Kg) biwk × 2 injections; $B53_2$-Fc + 4-1BB (5 mg/Kg and 1 mg/Kg, respectively) biwk × 2; PD-1 antibody (5 mg/Kg) biwk × 2; and $B53_2$-Fc + PD-1 (5 mg/Kg each) biwk × 2. Tumor volumes are expressed as mean ± SEM and analysed by two-way ANOVA followed by Bonferroni post-hoc analysis. Δtumor inhibition is calculated from the ΔT/ΔC tumor growth ratio, which represents the growth of the tumor in % from the beginning of the treatment (e.g. 300 mm^3), to the end volume of the treatment (e.g. 1000 mm^3) compared to isotype. biwk= twice a week.

Fig. 20 shows the immune contexture of Tumor Infiltrating Lymphocytes (TILs) analysis from Pan02 pancreatic cancer mice with large tumors treated with $B53_2$-Fc, 4-1BB and PD-1 by flow cytometry (continuation of experiment in Fig. 19). Relevant leukocytes analysed infiltrating the tumor: A) CD3+ T cells, CD4+ T cells, Regulatory T cells (Treg), CD8+ T cells, and the CD8+/Treg ratio. B) Granulocytes, Macrophages, Macrophage M1-type, Macrophage M2-type, and Myeloid Derived Suppressor Cells (MDSCs). C) M1/M2 macrophage ratio, Monocytes, Natural killer cells (NK), and Natural KillerT cells (NKT). Leukocytes % are expressed as box plots showing sample maxima and minima, and each group analysed by one-way ANOVA followed by Dunnet post-hoc analysis *p<0.05; **p<0.01; ***p<0.001; ****p<0.0001.

Fig. 21 shows the immune contexture of blood leukocyte analysis from treated Pan02 pancreatic cancer mice with large tumors treated with $B53_2$-Fc, 4-1BB and PD-1 by flow cytometry (continuation of experiment in Fig. 19). Relevant leukocytes analysed present in the blood: A) CD3+ T cells, CD4+ T cells, Regulatory T cells (Treg), CD8+ T cells, and CD8+/Treg ratio. B) Th1 cells (CD4+ T cells IFNy+), Natural Killer cells (NK), and Natural

Killer T cells (NKT). C) Monocytes, Granulocyte-Myeloid Derived suppressor cells (G-MDSCs), and Monocytic-Myeloid Derived Suppressor cells (M-MDSCs). Leukocytes % are expressed as box plots showing sample maxima and minima, and each group analysed by one-way ANOVA followed by Dunnet post-hoc analysis *p<0.05; **p<0.01; ***p<0.001; ****p<0.0001.

Fig. 22 shows the in vivo study of $B57_2$-Fc in combination with PD-1 and 4-1BB antibodies in large tumors of the pancreatic Pan02 syngeneic mouse model. A) Mean average tumor volume in mm^3 of $B57_2$-Fc treated animals (n=6). B) % Δtumor inhibition of treated mice groups compared to control. The experimental design of injection time points of substances was as follow: isotype (5 mg/Kg) biwk $\times$ 2; $B57_2$-Fc (5 mg/Kg) biwk $\times$ 2; 4-1BB antibody (1 mg/Kg) biwk $\times$ 2 injections; $B57_2$-Fc + 4-1BB (5 mg/Kg and 1 mg/Kg, respectively) biwk $\times$ 2; PD-1 antibody (5 mg/Kg) biwk $\times$ 2; and $B57_2$-Fc + PD-1 (5 mg/Kg each) biwk $\times$ 2. Tumor volumes are expressed as mean $\pm$ SEM and analysed by two-way ANOVA followed by Bonferroni post-hoc analysis. Δtumor inhibition is calculated from the $\Delta T/\Delta C$ tumor growth ratio, which represents the growth of the tumor in % from the beginning of the treatment (e.g. 300 mm^3), to the end volume of the treatment (e.g. 1000 mm^3) compared to isotype. biwk= twice a week.

Fig. 23 shows the immune contexture of Tumor Infiltrating Lymphocytes (TILs) analysis from Pan02 pancreatic cancer mice with large tumors treated with $B57_2$-Fc, 4-1BB and PD-1 by flow cytometry (continuation of experiment in Fig. 22). Relevant leukocytes analysed infiltrating the tumor: A) CD3+ T cells, CD4+ T cells, Regulatory T cells (Treg), CD8+ T cells, and the CD8+/Treg ratio. B) Granulocytes, Macrophages, Macrophage M1-type, Macrophage M2-type, and Myeloid Derived Suppressor Cells (MDSCs). C) M1/M2 macrophage ratio, Monocytes, Natural killer cells (NK), and Natural KillerT cells (NKT). Leukocytes % are expressed as box plots showing sample maxima and minima, and each group analysed by one-way ANOVA followed by Dunnet post-hoc analysis *p<0.05; **p<0.01; ***p<0.001; ****p<0.0001.

Fig. 24 shows the immune contexture of blood leukocyte analysis from treated Pan02 pancreatic cancer mice with large tumors treated with $B57_2$-Fc, 4-1BB and PD-1 by flow cytometry (continuation of experiment in Fig. 22). Relevant leukocytes analysed present in the blood: A) CD3+ T cells, CD4+ T cells, Regulatory T cells (Treg), CD8+ T cells, and CD8+/Treg ratio. B) Th1 cells (CD4+ T cells IFNy+), Natural Killer cells (NK), and Natural Killer T cells (NKT). C) Monocytes, Granulocyte-Myeloid Derived suppressor cells (G-MDSCs), and Monocytic-Myeloid Derived Suppressor cells (M-MDSCs). Leukocytes % are expressed as box plots showing sample maxima and minima, and each group analysed by one-way ANOVA followed by Dunnet post-hoc analysis *p<0.05; **p<0.01; ***p<0.001; ****p<0.0001.

Fig. 25 shows the in vivo study of $B58_2$-Fc in combination with PD-1 and 4-1BB antibodies in large tumors of the pancreatic Pan02 syngeneic mouse model. A) Mean average tumor volume in mm^3 of $B58_2$-Fc treated animals (n=6). B) % Δtumor inhibition of treated mice groups compared to control. The experimental design of injection time points of substances was as follow: isotype (5 mg/Kg) biwk $\times$ 2; $B58_2$-Fc (5 mg/Kg) biwk $\times$ 2; 4-1BB antibody (1 mg/Kg) biwk $\times$ 2 injections; $B58_2$-Fc + 4-1BB (5 mg/Kg and 1 mg/Kg, respectively) biwk $\times$ 2; PD-1 antibody (5 mg/Kg) biwk $\times$ 2; and $B58_2$-Fc + PD-1 (5 mg/Kg each) biwk $\times$ 2. Tumor volumes are expressed as mean $\pm$ SEM and analysed by two-way ANOVA followed by Bonferroni post-hoc analysis. Δtumor inhibition is calculated from the $\Delta T/\Delta C$ tumor growth ratio, which represents the growth of the tumor in % from the beginning of the treatment (e.g. 300 mm^3), to the end volume of the treatment (e.g. 1000 mm^3) compared to isotype. biwk= twice a week.

Fig. 26 shows the immune contexture of Tumor Infiltrating Lymphocytes (TILs) analysis from Pan02 pancreatic cancer mice with large tumors treated with $B58_2$-Fc, 4-1BB and PD-1 by flow cytometry (continuation of experiment in Fig. 25). Relevant leukocytes analysed infiltrating the tumor: A) CD3+ T cells, CD4+ T cells, Regulatory T cells (Treg), CD8+ T cells, and the CD8+/Treg ratio. B) Granulocytes, Macrophages, Macrophage M1-type, Macrophage M2-type, and Myeloid Derived Suppressor Cells (MDSCs). C) M1/M2 macrophage ratio, Monocytes, Natural killer cells (NK), and Natural KillerT cells (NKT). Leukocytes % are expressed as box plots showing sample maxima and minima, and each group analysed by one-way ANOVA followed by Dunnet post-hoc analysis *p<0.05; **p<0.01; ***p<0.001; ****p<0.0001.

Fig. 27 shows the immune contexture of blood leukocyte analysis from treated Pan02 pancreatic cancer mice with large tumors treated with $B58_2$-Fc, 4-1BB and PD-1 by flow cytometry (continuation of experiment in Fig. 25). Relevant leukocytes analysed present in the blood: A) CD3+ T cells, CD4+ T cells, Regulatory T cells (Treg), CD8+ T cells, and CD8+/Treg ratio. B) Th1 cells (CD4+ T cells IFNy+), Natural Killer cells (NK), and Natural

Killer T cells (NKT). C) Monocytes, Granulocyte-Myeloid Derived suppressor cells (G-MDSCs), and Monocytic-Myeloid Derived Suppressor cells (M-MDSCs). Leukocytes % are expressed as box plots showing sample maxima and minima, and each group analysed by one-way ANOVA followed by Dunnet post-hoc analysis *p<0.05; **p<0.01; ***p<0.001; ****p<0.0001.

Fig. 28 shows the in vivo study of $C08_2$-Fc in combination with PD-1 and 4-1BB antibodies in large tumors of the pancreatic Pan02 syngeneic mouse model. A) Mean average tumor volume in mm^3 of $C08_2$-Fc treated animals (n=6). B) % Δtumor inhibition of treated mice groups compared to control. The experimental design of injection time points of substances was as follow: isotype (5 mg/Kg) biwk × 2; $C08_2$-Fc (5 mg/Kg) biwk × 2; 4-1BB antibody (1 mg/Kg) biwk × 2 injections; $C08_2$-Fc + 4-1BB (5 mg/Kg and 1 mg/Kg, respectively) biwk × 2; PD-1 antibody (5 mg/Kg) biwk × 2; and $C08_2$-Fc + PD-1 (5 mg/Kg each) biwk × 2. Tumor volumes are expressed as mean ± SEM and analysed by two-way ANOVA followed by Bonferroni post-hoc analysis. Δtumor inhibition is calculated from the ΔT/ΔC tumor growth ratio, which represents the growth of the tumor in % from the beginning of the treatment (e.g. 300 mm^3), to the end volume of the treatment (e.g. 1000 mm^3) compared to isotype. biwk= twice a week.

Fig. 29 shows the immune contexture of Tumor Infiltrating Lymphocytes (TILs) analysis from Pan02 pancreatic cancer mice with large tumors treated with $C08_2$-Fc, 4-1BB and PD-1 by flow cytometry (continuation of experiment in Fig. 28). Relevant leukocytes analysed infiltrating the tumor: A) CD3+ T cells, CD4+ T cells, Regulatory T cells (Treg), CD8+ T cells, and the CD8+/Treg ratio. B) Granulocytes, Macrophages, Macrophage M1-type, Macrophage M2-type, and Myeloid Derived Suppressor Cells (MDSCs). C) M1/M2 macrophage ratio, Monocytes, Natural killer cells (NK), and Natural KillerT cells (NKT). Leukocytes % are expressed as box plots showing sample maxima and minima, and each group analysed by one-way ANOVA followed by Dunnet post-hoc analysis *p<0.05; **p<0.01; ***p<0.001; ****p<0.0001.

Fig. 30 shows the immune contexture of blood leukocyte analysis from treated Pan02 pancreatic cancer mice with large tumors treated with $C08_2$-Fc, 4-1BB and PD-1 by flow cytometry (continuation of experiment in Fig. 28). Relevant leukocytes analysed present in the blood: A) CD3+ T cells, CD4+ T cells, Regulatory T cells (Treg), CD8+ T cells, and CD8+/Treg ratio. B) Th1 cells (CD4+ T cells IFNy+), Natural Killer cells (NK), and Natural Killer T cells (NKT). C) Monocytes, Granulocyte-Myeloid Derived suppressor cells (G-MDSCs), and Monocytic-Myeloid Derived Suppressor cells (M-MDSCs). Leukocytes % are expressed as box plots showing sample maxima and minima, and each group analysed by one-way ANOVA followed by Dunnet post-hoc analysis *p<0.05; **p<0.01; ***p<0.001; ****p<0.0001.

Fig. 31 shows the in vivo study of $C12_2$-Fc in combination with PD-1 and 4-1BB antibodies in large tumors of the pancreatic Pan02 syngeneic mouse model. A) Mean average tumor volume in mm^3 of $C12_2$-Fc treated animals (n=6). B) % Δtumor inhibition of treated mice groups compared to control. The experimental design of injection time points of substances was as follow: isotype (5 mg/Kg) biwk × 2; $C12_2$-Fc (5 mg/Kg) biwk × 2; 4-1BB antibody (1 mg/Kg) biwk × 2 injections; $C12_2$-Fc + 4-1BB (5 mg/Kg and 1 mg/Kg, respectively) biwk × 2; PD-1 antibody (5 mg/Kg) biwk × 2; and $C12_2$-Fc + PD-1 (5 mg/Kg each) biwk × 2. Tumor volumes are expressed as mean ± SEM and analysed by two-way ANOVA followed by Bonferroni post-hoc analysis. Δtumor inhibition is calculated from the ΔT/ΔC tumor growth ratio, which represents the growth of the tumor in % from the beginning of the treatment (e.g. 300 mm^3), to the end volume of the treatment (e.g. 1000 mm^3) compared to isotype. biwk= twice a week.

Fig. 32 shows the immune contexture of Tumor Infiltrating Lymphocytes (TILs) analysis from Pan02 pancreatic cancer mice with large tumors treated with $C12_2$-Fc, 4-1BB and PD-1 by flow cytometry (continuation of experiment in Fig. 31). Relevant leukocytes analysed infiltrating the tumor: A) CD3+ T cells, CD4+ T cells, Regulatory T cells (Treg), CD8+ T cells, and the CD8+/Treg ratio. B) Granulocytes, Macrophages, Macrophage M1-type, Macrophage M2-type, and Myeloid Derived Suppressor Cells (MDSCs). C) M1/M2 macrophage ratio, Monocytes, Natural killer cells (NK), and Natural KillerT cells (NKT). Leukocytes % are expressed as box plots showing sample maxima and minima, and each group analysed by one-way ANOVA followed by Dunnet post-hoc analysis *p<0.05; **p<0.01; ***p<0.001; ****p<0.0001.

Fig. 33 shows the immune contexture of blood leukocyte analysis from treated Pan02 pancreatic cancer mice with large tumors treated with $C12_2$-Fc, 4-1BB and PD-1 by flow cytometry (continuation of experiment in Fig. 31). Relevant leukocytes analysed present in the blood: A) CD3+ T cells, CD4+ T cells, Regulatory T cells (Treg), CD8+ T cells, and CD8+/Treg ratio. B) Th1 cells (CD4+ T cells IFNy+), Natural Killer cells (NK), and Natural

Killer T cells (NKT). C) Monocytes, Granulocyte-Myeloid Derived suppressor cells (G-MDSCs), and Monocytic-Myeloid Derived Suppressor cells (M-MDSCs). Leukocytes % are expressed as box plots showing sample maxima and minima, and each group analysed by one-way ANOVA followed by Dunnet post-hoc analysis *p<0.05; **p<0.01; ***p<0.001; ****p<0.0001.

Examples

[0060] The inventors surprisingly found that MHC-Ia open conformers interact with diverse immunoregulatory cell surface receptors present in NK cells, NKT cells, T cells, macrophages and MDSC cells with unique binding or stronger affinity than their control MHC-Ia heterotrimers. HLA class I-a open conformers can be used as a therapeutics to target diseases were white blood cells impair the development of protective immunity, as is the case of cancer and infectious diseases.

[0061] Additionally, they discovered a novel in vivo mode of action with injections of HLA_2-Fc as monotherapy or combinatorial approaches using checkpoint modulatory agents. HLA_2-Fc therapy alone or in combinatorial therapies can modulate the infiltration of diverse sets of leukocytes into tumors as determined by the increased infiltration of macrophages M1/M2 ratio, increased NK cells, NKT cells, CD3+ T cells, and CD8+T cells, and reduction of MDSCs.

[0062] Furthermore, they observed that systemically by blood analysis HLA_2-Fc therapy increase the expansion of NKT cells and in some cases Th1 cells, indicating the presence of a biomarker that can be used for therapy efficacy in pre-clinical and clinical settings. Interestingly, they also observed that monotherapy with 4-1BB increases systemically the expansion of CD3+, CD4+, CD8+ T cells and Tregs in the blood of animals, indicating a potential side effect of hyper activation of the immune system by 4-1BB. Diverse combinations of HLA_2-Fc + 4-1BB reduced significantly the presence of blood CD3+, CD4+, Treg, and CD8+ T cells, indicating a positive combinatorial approach in case of unwanted lymphocyte expansion on the blood of treated patients with agonistic antibodies.

[0063] Overall, the mode of action of MHC-Ia open conformers, particularly when present as fusion proteins comprising an Fc immunoglobulin fragment, alone or in a combinatorial approach with antagonistic/agonistic antibodies is of undoubted relevance as immunomodulatory agents, and can be useful for its translation in the treatment of cancer.

[0064] HLA open conformers can be used as a therapeutic to target diseases where immunomodulation is a therapeutic approach, as is the case of cancer and infectious diseases.

In vitro tests

[0065] MHC-Ia open conformers bind to immunoregulatory receptors expressed in diverse types of white blood cells with unique binding or different affinity than their HLA-β2m-Fc control counterparts

[0066] The inventors determined if MHC-Ia open conformers interact with specific immunomodulatory receptors by enzyme-linked immunosorbent assay (ELISA). Results demonstrated that MHC-Ia open conformers interact uniquely to KIR3DL2, and PTPRJ (for exception of HLA-C-β2m-Fc) and display different affinities to KIR3DL1, KIR3DL3, LILRB1, LILRB2, and Pirb immmunoregulatory receptors than their HLA-β2m-Fc control counterparts (Fig. 5 A-G). This data shows for the first time that MHC classical alleles (HLA-A, HLA-B & HLA-C) (MHC-Ia) have a similar binding pattern to immunoregulatory receptors when they are present as open conformers.

MHC-Ia open conformers blocks conversion of murine CD4$^+$ T cells into iTregs

[0067] The influence of MHC-Ia molecules on naive CD4$^+$ T cells for iTreg conversion was analysed with 10 μg/mL of HLA_2-Fc ($A25_2$-Fc, $A30_2$-Fc, $B27_2$-Fc, $B53_2$-Fc, $B57_2$-Fc, $B58_2$-Fc, $C08_2$-Fc and $C12_2$-Fc), HLA-β2m controls (A25-β2m-Fc, A30-β2m-Fc, B27-β2m-Fc, B53-β2m-Fc, B57-β2m-Fc, B58-β2m-Fc, C08-β2m-Fc and C12-β2m-Fc), isotype, and PBS, incubated with naive CD4$^+$ T cells in optimal culture conditions for iTreg conversion. MHC-Ia open conformers demonstrated invariably to down modulate the induction of FoxP3 (Fig. 6) and thus conversion of naive CD4+ T cells into iTregs.

[0068] *MHC-Ia open conformers impair the proliferation of leukaemia T cells.*

[0069] The inventors determined the effect of MHC-Ia open conformers ($A25_2$-Fc, $A30_2$-Fc, $B27_2$-Fc, $B53_2$-Fc, $B57_2$-Fc, $B58_2$-Fc, $C08_2$-Fc and $C12_2$-Fc) with the blocking of proliferation in different tumor cell lines. Results demonstrated that MHC-Ia open conformers modulate invariably the proliferation of lymphoma T cell lines, when compared to their control counterparts HLA-β2m-Fc (Fig. 7) or isotype IgG4 (data not provided), indicating its potential application to the treatment of lymphoma as a targeted therapy.

In vivo tests

[0070] The *in vivo* proof of concept of MHC-Ia open conformers as immunomodulatory therapeutic molecules for

cancer therapy was demonstrated using a validated pre-clinical syngeneic murine C38 and MC38-OVA colon carcinoma models (Figure 8 and 9), and in the pancreatic (Pan02) cancer mouse model (Figure 10, 13, 16, 19, 22, 25, 28 and 31).

Production of MHC-Ia open conformers as a human Fc fusion protein in CHO cells

[0071] A valid strategy, from a therapeutic point of view, is to produce MHC-Ia open conformers molecules in stable format (Fc fusion), to increase solubility, stability, avidity, half-life, and from a technological point of view, cost-effective production and purification in mammalian systems. HLA-β2m-Fc complex was successfully produced by inserting the alpha 1, 2 and 3 domains of HLA-A25, HLA-A30, HLA-B27, HLA-B53, HLA-B57, HLA-B58, HLA-C08 and HLA-C12 into a human IgG4-Fc vector cassette (Fig. 3A), together with a human-β2m vector, necessary for extracellular production of the HLA-β2m-Fc protein (Fig. 3A,B). Transfections in Chinese hamster ovary cells (CHO) cells were performed using both the HLA-Fc-vector + β2m-vector at a ratio of 1:1. Supernatants were collected and HLA-β2m-Fc purified using standard antibody purification protocols (Recombinant Protein Purification Handbook, principles and methods. 2009. GE Healthcare, 18-1142-75). Separation of β2m from HLA-Fc free-heavy chains was performed using denaturing conditions by SEC (Fig. 4A), or dialysis (data not shown). Refolding of HLA_2-Fc was assessed using the dilution method in refolding buffer and analysed SDS page (Fig. 4B,C) or by western blot (data not shown).

Pre-clinical combination therapy tests of HLA_2-Fc with CTLA4 and PD-1 antibodies in murine syngeneic colon cancer models

[0072] The *in vivo* proof of concept study using HLA_2-Fc ($A30_2$-Fc, $B27_2$-Fc, $B57_2$-Fc, $B58_2$-Fc, and $C08_2$-Fc) as immunomodulatory therapeutic molecules was demonstrated in the C38 and MC38-OVA murine colon carcinoma cancer models as monotherapy or in combination with a murine CTLA4 or murine PD-1 antibody.

[0073] Following established protocols C38 or MC38-OVA fragment tumours were subcutaneously injected in the flank of syngeneic mice. Once the tumour reached 60 mm^3 (between 1-2 weeks after transplantation of tumors), mice were distributed according to their tumor volume. $A30_2$-Fc, $B27_2$-Fc, $B57_2$-Fc, $B58_2$-Fc, and $C08_2$-Fc was injected i.p. six times every 3rd day (Q3Dx6), CTLA4 was injected two times (Q3Dx2), and PD-1 injected 4 times twice a week (biwk × 2) (Fig. 8A).

[0074] Selected HLA_2-Fc can synergize and enhance anti-tumor responses in syngeneic C38 and MC38-OVA colon cancer mouse models (Fig. 8 & 9) either as monotherapy ($C08_2$-Fc) (Fig. 8D) or in combination with checkpoint antibodies, such as PD-1 + $A30_2$-Fc (Fig. 8B), $B58_2$-Fc (Fig. 8C), $B57_2$-Fc (Fig. 9B) and CTLA4 + $B27_2$-Fc (Fig. 9A).

Pre-clinical combination therapy tests of HLA_2-Fc with PD-1 and 4-1BB antibodies in large tumors of a murine syngeneic Pancreatic cancer model

[0075] For the pancreas (Pan02) cancer mouse model, following established protocols Pan02 cells were injected at 1×10^5 in the right flank of syngeneic mice respectively. Once the tumors had reached 300 mm3 (approximately 3 weeks after injection of cells) mice were statistically distributed according to their tumor volume. To note that large tumors are harder to treat than smaller tumors, but are useful for further analysis of tumor infiltrating lymphocytes (TILs). Furthermore large tumors are closer to a clinical setting where interventions with immunomodulators are performed in large size tumors of patients.

[0076] In pancreas (Pan02) data demonstrated that HLA_2-Fc combination with PD-1 antibody can significantly reduce large Pan02 tumors in combination with $A25_2$-Fc (Fig. 10A-B), $B27_2$-Fc (Fig. 16A-B), $C08_2$-Fc (Fig. 28A-B), and $C12_2$-Fc (Fig. 31A-B), whereas PD-1 monotherapy showed no therapeutic effect. Other HLA_2-Fc combinations with PD-1 did not demonstrate statistical significance, however % Δtumor inhibition was observed in combination $B57_2$-Fc (Fig. 22). Additionally, combo therapy of HLA_2-Fc with 4-1BB antibody demonstrated to significantly reduce the tumor size or several HLA_2-Fc combo therapies (for exception of $A30_2$-Fc and $C08_2$-Fc) when compared to isotype. The most striking tumor reductions ($p<0.01$) were observed with $B53_2$-Fc (Fig. 19A-B), $B57_2$-Fc (Fig. 22A-B), and $B58_2$-Fc (Fig. 25A-B). 4-1BB monotherapy was not significantly different when compared to isotype control. Monotherapy with $C08_2$-Fc (Fig. 28A-B) showed significant tumor reduction ($p<0.01$) compared to isotype.

[0077] The tumor immune contexture of pancreas (Pan02) mice demonstrated the influence of HLA_2-Fc therapy towards diverse sets of tumor infiltrating leukocytes as observed with the infiltration of macrophages M1/M2 ratio, increased NK cells, NKT cells, CD3+ T cells, and CD8+T cells, and reduction of MDSCs, with variations for each HLA_2-Fc as observed in $A25_2$-Fc (Fig. 11A-C), $A30_2$-Fc (14A-C), $B27_2$-Fc (17A-C), $B53_2$-Fc (20A-C), $B57_2$-Fc (23A-C), $B58_2$-Fc (26A-C), $C08_2$-Fc (29A-C), and $C12_2$-Fc (32A-C). Systemically analysis of leukocytes from the blood demonstrated only few changes when compared to their control monotherapy counterparts in NKT cells and Th1 cells for some cases, $A25_2$-Fc (Fig. 12A-C), $A30_2$-Fc (15A-C), $B27_2$-Fc (18A-C), $B53_2$-Fc (21A-C), $B57_2$-Fc (24A-C), $B58_2$-Fc (27A-C), $C08_2$-Fc (30A-C), and $C12_2$-Fc (33A-C).

Conclusion

[0078] The present invention demonstrates for the first time that the family of classical MHC-Ia molecules when produced as heavy chains without β2m (HLA-A, HLA-B and HLA-C open conformers and their corresponding HLA_2-Fc fusion proteins) have immunomodulatory properties that differ from their control HLA-β2m counterparts. Using as non-limiting examples diverse sets of HLA alleles the inventors provide data demonstrating that invariably MHC-Ia molecules, when present as open conformers are immunomodulatory agents with unique properties as demonstrated by the modulation of leukocytes present in the tumor microenvironment and in the blood. Furthermore its use is not only limited to modulatory agents, but also for its use as therapeutics for the treatment of cancer as demonstrated in pre-clinical cancer mouse models of colon cancer and pancreatic cancer either as monotherapy or in combination therapy with checkpoint inhibitor antibodies (e.g. CTLA4 and PD-1) and checkpoint agonistic antibodies (e.g. 4-1BB).

[0079] Interaction of HLA_2-Fc with diverse immunoregulatory receptors (KIR3DL1, KIR3DL2, KIR3DL3, LILRB1, LILRB2, PTPRJ and Pirb) distributed in diverse white blood cells (e.g. NK, NKT, CD4+ T-cells, macrophages and MDSCs) demonstrates that the multitasking nature of the molecules paves a new way of modulating the immune system with HLA open conformers.

[0080] Additionally, HLA_2-Fc molecules demonstrated to block the conversion of naive CD4+ T-cells to iTregs *in vitro,* pointing out to a mode of action were HLA_2-Fc acts as an immunomodulatory molecule affecting the differentiation and function of iTregs. Targeting iTregs is a strategy for diverse therapeutic indications, such as infectious diseases and cancer.

[0081] Overall, the mode of action of HLA_2-Fc as combinatorial approaches with antagonistic/agonistic antibodies is of undoubted relevance in the treatment of cancer, and correlates to the current clinical need in cancer immunotherapy.

[0082] HLA_2-Fc molecules emerge as a novel class of immunomodulatory drugs. *In vitro* and *in vivo* data points to a mechanism were HLA_2-Fc molecules act as a switch-on mechanism for the activation of anti-tumor immunity. Without wishing to be bound by theory, the inventors hypothesize that the interaction of HLA_2-Fc open conformers with diverse immunomodulatory receptors present in NK, T cells, macrophages and MDSCs, and functional modulation of Tregs participate synergistically and exacerbates the immune response.

Materials and Methods

Cell lines

[0083] *In vivo* experiments were performed using C38 and MC38-OVA colon carcinoma mouse cell lines.

[0084] *In vitro* experiment cell lines used were: EL4, mouse T cell lymphoma; EG.7, mouse T cell lymphoma; Jurkat, human T cell lymphoma; L428, human Hodgkin lymphoma; L540, human Hodgkin lymphoma; L1236, human Hodgkin lymphoma; Daudi, B cell lymphoma; IMR-5, neuroblastoma; SK-N-AS, neuroblastoma; and M130428, Melanoma.

Antibodies

[0085] Lymphocytes populations for iTreg conversion experiments were stained with: CD4 (FITC-BD Bioscience), FoxP3+ (efluor 450- eBioscience), CD3 (PE-Cy7- eBioscience), CD45 (PerCP-eBioscience).

[0086] Analysis of tumor infiltrating lymphocytes was performed with the following antibodies: CD45 (Biolegend, clone 30-F11); CD3 (BD Bioscience, clone 145-2011); CD4 (Biolegend, clone GK1.5), CD8 (BD Bioscience, clone 53-6.7), CD25 (Biolegend, clone PC61), FoxP3 (eBioscience, clone FJK-16s), CD335 (Biolegend, clone 29A1.4), F4/80 (Biolegend, clone BM8), CD11b (Biolegend, clone M1/70), Gr-1 (BD Bioscience, clone RB6-8C5), MHCII I-A/I-E (BD Bioscience, clone 2G9), CD206 (Biolegend, clone C068C2) and LID stain (eBioscience).

[0087] Analysis of blood leukocytes was performed with the following antibodies: CD45 (Biolegend, clone 30-F11); CD3 (BD Bioscience, clone 145-2011), CD4 (Biolegend, clone GK1.5), CD8 (BD Bioscience, clone 53-6.7), FoxP3 (eBioscience, clone FJK-16s), T-Bet (BD Bioscience, clone 4B10), CD335 (Biolegend, clone 29A1.4), F4/80 (Biolegend, clone BM8), CD115 (Biolegend, clone AFS98), CD11b (Biolegend, clone M1/70), Ly6G (Biolegend, clone 1A8), Ly6C (Biolegend, clone HK1.4) and LID stain (eBioscience).

[0088] Checkpoint inhibitor antibodies CTLA4 clone 9H10, PD-1 clone RMP1-14, and agonist antibody 4-1BB clone 3H3 were obtained from Bio X Cell Co.

[0089] HC10 mAb (IgG2a) binding to β2m-free heavy chains of MHC-Ia alleles was a gift from Dr. Hidde Ploegh (MIT, MA).

Production, purification and re-folding of HLA_2-Fc

[0090] Recombinant production of HLA-β2m-Fc (A25-β2m-Fc, A30-β2m-Fc, B2705-β2m-Fc, B53-β2m-Fc, B57-β2m-

Fc, B58-β2m-Fc, C08-β2m-Fc & C12-β2m-Fc) was achieved by inserting the alpha 1, 2 and 3 domains of HLAs into a human IgG4-Fc vector (InvivoGen), and the human β2-microglobulin (β2m) in a separate vector. Production of recombinant HLA-β2m-Fc was performed by co-transfection of the HLA-Fc-vector sand β2m-vector into Chinese hamster ovary (CHO) cells. Production of HLA-β2m-Fc was outsourced to Evitria AG.

[0091] Purification of HLA-β2m-Fc constructs was performed using conventional protocols for antibody purification. Production of HLA_2-Fc was performed with the addition of a denaturing step to remove β2m from the HLA-β2m-Fc complex.

[0092] Briefly, the capture step of HLA-β2m-Fc proteins was performed after running supernatants (5 mL/min) through protein-G columns (Amersham Pharmacia). Intermediate purification steps were performed by eluting the selected HLA-β2m-Fc from protein G-columns using elution buffer (100 mM glycine, pH 2.0), and recovering fractions in 8M Urea, 100 mM Tris-HCl pH 8.0. The 1st Polishing step was to separate HLA-Fc monomers fractions from β2m by either size exclusion chromatography (SEC) using superdex 200 prep grade or Sephacryl S-100 HR (GE Lifescience) with an AKTA system (GE Lifescience), or by dialysis with membranes of 50 KDa pore size (Millipore). The recovered HLA-Fc monomers from both protocols were refolded by the dilution method after pulsation of the HLA-Fc monomers at 3 times with intervals of 8 hours each in 100 times volume of refolding buffer (50 mM Tris-HCl pH8.5, 500 mM L-Arginine, 1 mM EDTA, 0.15 mM NaCl, 1% Sucrose, 0.01% Tween-20). The 2nd Polishing step by SEC was performed to remove further impurities and to buffer exchange newly recovered fractions of HLA_2-Fc proteins into dilution buffer (PBS, 1% Sucrose, and 0.01% Tween-20). Purified solutions of HLA_2-Fc proteins ($A25_2$-Fc, $A25_2$-Fc, $B2705_2$-Fc, $B53_2$-Fc, $B57_2$-Fc, $B58_2$-Fc, $C08_2$-F, $C12_2$-Fc) were filter sterilized using 0.2 μm membranes (Millipore).

[0093] Fractions HLA-β2m-Fc complexes and HLA_2-Fc were analysed by gradient 4-20% SDS polyacrylamide gel electrophoresis (SDS-PAGE) and western blot using HC10 (specific for HLA-free-heavy chains) antibodies. β2m western blots were performed with and without denaturing conditions (10 mM DTT) (data not shown).

ELISA Assays

[0094] Competition ELISA assays were performed using Maxisorp (Nunc, Switzerland) 96 well plates coated with 10 μg/mL of selected leukocyte receptors (human KIR3DL1, human KIR3DL2, human KIR3DL3, human LILRB1, human LILRB2, human PTPRJ and mouse Pirb) purchased from Creative Biomart. Receptors were incubated for ON 4°C, blocked with 5% milk powder-TBS 2 hrs. HLA_2-Fc selected constructs ($A25_2$-Fc, $A30_2$-Fc, $B2705_2$-Fc, $B53_2$-Fc, $B57_2$-Fc, $B58_2$-Fc, $C08_2$-F, and $C12_2$-Fc) and their controls (A25-β2m-Fc, A30-β2m-Fc, B2705-β2m-Fc, B53-β2m-Fc, B57-β2m-Fc, B58-β2m-Fc, C08-β2m-Fc and C12-β2m-Fc) and isotype IgG4 were added at 10 μg/mL for 2 hrs RT. HRP-conjugated antibodies against human Fc were used as detectors.

Flow cytometry of leukocytes

[0095] Flow cytometry analysis was performed using a FACS canto II (BD Bioscience) and data were analysed using FlowJo version 7.6.4.

Generation of Tregs

[0096] To induce expression of Foxp3 in murine $CD4^+$ T cells, we harvested spleen cells from C57BL/6 splenocytes and purified (Mouse Naive $CD4^+$ T Cell Isolation Kit- Easy Sep) to obtain $CD4^+$ T naive cells. Cells were then cultured for 96 h at 10^5 cells/200 μL/well in 96-well plates with coated 5 μg/mL anti-CD3mAb (eBioscience), soluble 2 μg/mL anti-CD28 mAb (Biolegend), 10 μg/mL of TGF-β1 (R&D systems) and 100 IU/mL of IL-2 (R&D systems).

iTreg conversion in the presence of HLA_2-Fc

[0097] Murine naive $CD4^+$ T cells in optimal culture conditions for iTreg conversion were incubated in the presence at dose concentrations (5μg/200 μL) of HLA_2-Fc ($A25_2$-Fc, $A30_2$-Fc, $B2705_2$-Fc, $B53_2$-Fc, $B57_2$-Fc, $B58_2$-Fc, $C08_2$-F, and $C12_2$-Fc), controls (A25-β2m-Fc, A30-β2m-Fc, B2705-β2m-Fc, B53-β2m-Fc, B57-β2m-Fc, B58-β2m-Fc, C08-β2m-Fc and C12-β2m-Fc) Isotype IgG4, media without differentiation factors and PBS for 72 h. iTreg conversion was measured by flow cytometry.

Proliferation assay

[0098] Cells were plated in round 96-wells plates at a density of 5×10^5 cells / well following the addition of drugs at different concentrations (25, 10, and 5 μg/well) for 1 day. XTT proliferation assay was performed accordingly to the manual instructions (cell proliferation kit II, Roche). Results were obtained with the absorbance of wells at 450 nm using

a microtiter plate reader.

In vivo treatments

[0099] C38 or MC38-OVA tumour fragments were injected subcutaneously into the right flanks of syngeneic female C57BL/6 mice at week 6. Pan02 cell lines were injected at 1×10^5 in the right flank of syngeneic mice C57BL/6 at week 6. Animals were distributed according to their individual tumour volume size and divided into groups displaying no statistical differences between them. For C38 and MC38-OVA experimental treatment began when the tumors had reach $\pm$60 mm^3. For pancreas Pan02 experimental treatment began in large tumors of 300 mm^3. Tumour diameters were measured using a caliper, and volume was calculated according to the formula, $D/2\times d^2$ where *D* and *d* are the longest and shortest diameter of the tumour in mm, respectively.

[0100] The Experimental design of injection of substances was established as follow for colon (C38 and MC38): vehicle (PBS 200 μL) Q3Dx6; isotype (10mg/Kg) Q3Dx6; HLA_2-Fc (10 mg/Kg) Q3Dx6; anti-CTLA4 Q3Dx2 (1st injection 100 μg and 2nd injection 50 μg); PD-1 biwk $\times$ 2 (200 μg); HLA_2-Fc + CTLA-4 (Q3Dx6 and Q3Dx2, respectively); HLA_2-Fc + PD-1 (Q3Dx6 and biwk $\times$ 2, respectively). For pancreas (Pan02) the experimental design of injection of substances was as follow: isotype (5 mg/Kg) biwk $\times$ 2; HLA_2-Fc (5 mg/Kg) biwk $\times$ 2; PD-1 (5 mg/Kg) biwk $\times$ 2; 4-1BB (1 mg/Kg) biwk $\times$ 2; HLA_2-Fc + PD-1 biwk $\times$ 2; and HLA_2-Fc + 4-1BB biwk $\times$ 2.

[0101] *% ΔInhibition is calculated from the* $\Delta T/\Delta C$ tumor growth ratio, which represents the growth of the tumor in % from the beginning of the treatment (e.g. 300 mm3), to the end volume of the treatment (e.g. 1000 mm3) compared to control using the following formula: Mean % ΔInhibition = (mean(C)-mean(C0)) - (mean(T)-mean(T0)) / (mean(C)-mean(C0)) * 100%. Where T = treated group value, T0 - treated group initial value; C - control group value, C0 - control group initial value.

[0102] For the analysis of tumor infiltrating lymphocytes the following gating strategies where used: CD45+ for total leukocytes; CD45+ CD3+ for total T cells; CD45+ CD3+ CD4+ for CD4 T helper cell; CD45+ CD3+ CD8+ for CD8 Cytotoxic T cell; CD45+ CD3+ CD4+ FoxP3+ CD25+ for Treg cell; CD45+ CD3- CD11+ Gr-1+ for MDSCs; CD45+ CD3- CD11+ F4/80+ for Macrophages; CD45+ CD3- CD11+ F4/80+ MHCII+ for M1-type macrophages; CD45+ CD3-CD11+ F4/80+ CD206+ for M2-type macrophages; CD45+ Gr-1- F4/80- CD335+ for NK cells; and CD45+ Gr-1- F4/80- CD335+ CD3+ for NKT cells.

[0103] For the analysis of blood leukocytes the following gating strategies where used: CD45+ for total leukocytes; CD45+ CD3+ for total T cells; CD45+ CD3+ CD4+ for CD4 T helper cell; CD45+ CD3+ CD8+ for CD8 Cytotoxic T cell; CD45+ CD3+ CD4+ FoxP3+ for Treg cell; CD45+ CD3+ CD4+ T-Bet+ for Th1 cells; CD45+ CD3- CD11+ Ly6C+ Ly6G+ for G-MDSCs and M-MDSCs; CD45+ Ly6C- Ly6G- CD335+ for NK cells; and CD45+ Ly6C- Ly6G- CD335+ CD3+ for NKT cells.

[0104] Preparation of tumor and blood samples for flow cytometry were performed using protocols described by eBioscience (https://www.ebioscience.com/media/pdf/best-protocols/cell-preparation-for-flow-cytometry.pdf, accessed Feb 21, 2017).

Table 1: List of MHC-Ia alleles

HLA-A	HLA-B		HLA-C
A*01	B*07	B*53	C*01
A*02	B*08	B*54	C*02
A*03	B*13	B*55	C*03
A*11	B*14	B*56	C*04
A*23	B*15	B*57	C*05
A*24	B*18	B*58	C*06
A*25	B*27	B*59	C*07
A*26	B*35	B*67	C*08
A*29	B*37	B*73	C*12
A*30	B*38	B*78	C*14
A*31	B*39	B*81	C*15
A*32	B*40	B*82	C*16
A*33	B*42	B*83	C*17
A*34	B*44		C*18
A*36	B*46		
A*43	B*47		

(continued)

HLA-A	HLA-B	HLA-C
A*66	B*48	
A*68	B*49	
A*69	B*50	
A*74	B*51	
A*80	B*52	

Table 2: Selected MHC-Ia alleles

Sequence identifier (length in aa)	Amino acid sequence
A*25:01:01 HLA00071 (365aa) SEQ ID NO. 002	MAVMAPRTLVLLLSGALALTQTWAGSHSMRYFYTSVSRPGRGEPRFIAVGYVD DTQFVRFDSDAASQRMEPRAPWIEQEGPEYWDRNTRNVKAHSQTDRESLRIAL RYYNQSEDGSHTIQRMYGCDVGPDGRFLRGYQQDAYDGKDYIALNEDLRSWTA ADMAAQITQRKWETAHEAEQWRAYLEGRCVEWLRRYLENGKETLQRTDAPKTH MTHHAVSDHEATLRCWALSFYPAEITLTWQRDGEDQTQDTELVETRPAGDGTF QKWASVVVPSGQEQRYTCHVQHEGLPKPLTLRWEPSSQPTIPIVGIIAGLVLF GAVIAGAVVAAVMWRRKSSDRKGGSYSQAASSDSAQGSDMSLTACKV
A*30:01:01 HLA00089 (365aa) SEQ ID NO. 003	MAVMAPRTLLLLLSGALALTQTWAGSHSMRYFSTSVSRPGSGEPRFIAVGYVD DTQFVRFDSDAASQRMEPRAPWIEQERPEYWDQETRNVKAQSQTDRVDLGTLR GYYNQSEAGSHTIQIMYGCDVGSDGRFLRGYEQHAYDGKDYIALNEDLRSWTA ADMAAQITQRKWEAARWAEQLRAYLEGTCVEWLRRYLENGKETLQRTDPPKTH MTHHPISDHEATLRCWALGFYPAEITLTWQRDGEDQTQDTELVETRPAGDGTF QKWAAVVVPSGEEQRYTCHVQHEGLPKPLTLRWELSSQPTIPIVGIIAGLVLL GAVITGAVVAAVMWRRKSSDRKGGSYTQAASSDSAQGSDVSLTACKV
B*27:05:02 HLA00225 (362aa) SEQ ID NO. 004	MRVTAPRTLLLLLWGAVALTETWAGSHSMRYFHTSVSRPGRGEPRFITVGYVD DTLFVRFDSDAASPREEPRAPWIEQEGPEYWDRETQICKAKAQTDREDLRTLL RYYNQSEAGSHTLQNMYGCDVGPDGRLLRGYHQDAYDGKDYIALNEDLSSWTA ADTAAQITQRKWEAARVAEQLRAYLEGECVEWLRRYLENGKETLQRADPPKTH VTHHPISDHEATLRCWALGFYPAEITLTWQRDGEDQTQDTELVETRPAGDRTF QKWAAVVVPSGEEQRYTCHVQHEGLPKPLTLRWEPSSQSTVPIVGIVAGLAVL AVVVIGAVVAAVMCRRKSSGGKGGSYSQAACSDSAQGSDVSLTA
B*53:01:01 HLA00364 (362aa) SEQ ID NO. 005	MRVTAPRTVLLLLWGAVALTETWAGSHSMRYFYTAMSRPGRGEPRFIAVGYVD DTQFVRFDSDAASPRTEPRAPWIEQEGPEYWDRNTQIFKTNTQTYRENLRIAL RYYNQSEAGSHIIQRMYGCDLGPDGRLLRGHDQSAYDGKDYIALNEDLSSWTA ADTAAQITQRKWEAARVAEQLRAYLEGLCVEWLRRYLENGKETLQRADPPKTH VTHHPVSDHEATLRCWALGFYPAEITLTWQRDGEDQTQDTELVETRPAGDRTF QKWAAVVVPSGEEQRYTCHVQHEGLPKPLTLRWEPSSQSTIPIVGIVAGLAVL AVVVIGAVVATVMCRRKSSGGKGGSYSQAASSDSAQGSDVSLTA
B*57:01:01 HLA00381 (362aa) SEQ ID NO. 006	MRVTAPRTVLLLLWGAVALTETWAGSHSMRYFYTAMSRPGRGEPRFIAVGYVD DTQFVRFDSDAASPRMAPRAPWIEQEGPEYWDGETRNMKASAQTYRENLRIAL RYYNQSEAGSHIIQVMYGCDVGPDGRLLRGHDQSAYDGKDYIALNEDLSSWTA ADTAAQITQRKWEAARVAEQLRAYLEGLCVEWLRRYLENGKETLQRADPPKTH VTHHPISDHEATLRCWALGFYPAEITLTWQRDGEDQTQDTELVETRPAGDRTF QKWAAVVVPSGEEQRYTCHVQHEGLPKPLTLRWEPSSQSTVPIVGIVAGLAVL AVVVIGAVVAAVMCRRKSSGGKGGSYSQAACSDSAQGSDVSLTA

(continued)

Sequence identifier (length in aa)	Amino acid sequence
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C*08:01:01 HLA00445 (366aa) SEQ ID NO. 008	MRVMAPRTLILLLSGALALTETWACSHSMRYFYTAVSRPGRGEPRFIAVGYVD DTQFVQFDSDAASPRGEPRAPWVEQEGPEYWDRETQKYKRQAQTDRVSLRNLR GYYNQSEAGSHTLQRMYGCDLGPDGRLLRGYNQFAYDGKDYIALNEDLRSWTA ADTAAQITQRKWEAARTAEQLRAYLEGTCVEWLRRYLENGKKTLQRAEHPKTH VTHHPVSDHEATLRCWALGFYPAEITLTWQRDGEDQTQDTELVETRPAGDGTF QKWAAVVVPSGEEQRYTCHVQHEGLPEPLTLRWGPSSQPTIPIVGIVAGLAVL AVLAVLGAVMAVVMCRRKSSGGKGGSCSQAASSNSAQGSDESLIACKA
C*12:02:01 HLA00453 (366aa) SEQ ID NO. 009	MRVMAPRTLILLLSGALALTETWACSHSMRYFYTAVSRPGRGEPRFIAVGYVD DTQFVRFDSDAASPRGEPRAPWVEQEGPEYWDRETQKYKRQAQADRVSLRNLR GYYNQSEAGSHTLQRMYGCDLGPDGRLLRGYDQSAYDGKDYIALNEDLRSWTA ADTAAQITQRKWEAAREAEQWRAYLEGTCVEWLRRYLENGKETLQRAEHPKTH VTHHPVSDHEATLRCWALGFYPAEITLTWQRDGEDQTQDTELVETRPAGDGTF QKWAAVVVPSGEEQRYTCHVQHEGLPEPLTLRWEPSSQPTIPIVGIVAGLAVL AVLAVLGAVMAVVMCRRKSSGGKGGSCSQAASSNSAQGSDESLIACKA

Claims

1. A fusion MHC-Ia open conformer, wherein said fusion MHC-Ia open conformer comprises or essentially consists of a first monomer or a first and a second monomer, wherein

   a. said first monomer, or each of said first and second monomer independently of the other monomer, comprises an HLA heavy chain selected from C08, A25, B58, A30, B53, and C12, and
   b. wherein said first monomer, or each of said first and second monomer are covalently linked to an Fc polypeptide sequence,

   wherein the HLA-heavy chain only consists of the HLA alpha 1, 2 and 3 domains.

2. The fusion MHC-Ia open conformer according to claim 1, wherein the HLA heavy chain is C08.

3. The fusion MHC-Ia open conformer according to claim 1 or 2, wherein an amino acid linker joins the HLA-heavy chain and the Fc polypeptide sequence.

4. The fusion MHC-Ia open conformer according to any one of the preceding claims, wherein the first and the second monomer are the same.

5. The fusion MHC-Ia open conformer according to any one of the preceding claims, wherein the fusion MHC-Ia open conformer additionally comprises a peptide epitope fragment, particularly wherein the first and/or second monomer additionally comprises a peptide epitope fragment.

6. The fusion MHC-Ia open conformer according to any one of the preceding claims, wherein the Fc domain comprises heavy chain constant regions C_H2 and C_H3 selected from any one of immunoglobulin type G (IgG), type A (IgA), type D (IgD), type E (IgE) or type M (IgM).

7. The fusion MHC-Ia open conformer according to any one of the preceding claims, wherein the amino acid linker comprises 1 to 50, particularly 5 to 40, more particularly 10 to 30, even more particularly 15 to 25 amino acids, linking the HLA-heavy chain to the Fc domain as one single polypeptide chain.

8. The fusion MHC-Ia open conformer according to any one of the preceding claims for use as a medicament.

9. The fusion MHC-Ia open conformer according to any one of the preceding claims 1 to 9 for use

a. in the treatment or prevention of cancer, or
b. as an immunomodulatory agent, particularly in a treatment of an infectious disease, more particularly in the treatment of human immunodeficiency virus (HIV) infection, hepatitis A, hepatitis B, hepatitis C, influenza, respiratory syncytial virus (RSV) infection, measles, herpes and yellow fever.

10. A nucleic acid molecule, wherein said nucleic acid molecule encodes a fusion MHC-Ia open conformer monomer according to any one of claims 1 to 7.

11. A virus comprising the nucleic acid molecule of claim 10 under control of a promoter sequence operable in a mammalian cell, particularly in a human cell, particularly an adenovirus, adeno-associated virus, a herpes virus or a lentivirus.

12. An *in vitro* genetically modified host cell comprising the nucleic acid molecule of claim 10.

13. A combination medicament comprising

a. a fusion MHC-Ia open conformer as specified in any one of claims 1 to 7, and
b. a checkpoint modulatory agent selected from

i. a checkpoint inhibitory agent (CPI), particularly wherein said CPI is selected from:

- an inhibitor of the interaction of CTLA4 with either B7-1 (cd80) and/or B7-2 (cd86), more particularly a polypeptide ligand to CTLA-4 or to cd80 or to cd86;
- an inhibitor of the interaction of PD-1 with either PD-L1 and/or PD-L2, more particularly a polypeptide ligand to PD-1 or to PD-L1 or to PD-L2; and
- an inhibitory polypeptide ligand, particularly an antibody, of T cell immunoglobulin and mucin domain-containing 3 (TIM-3); and

ii. a checkpoint agonist agent, particularly a checkpoint agonist antibody selected to bind to and activate the tumor necrosis factor receptor 4-1BB, particularly a monoclonal antibody against 4-1BB.

particularly wherein said checkpoint modulatory agent is a polypeptide selected from an antibody, an antibody fragment, and an antibody-like molecule, and the polypeptide is selectively reactive to a checkpoint mediator selected from CTLA4, PD-1, CD80, CD86, PD-L1, PD-L2, TIM-3, 4-1BB and 4-1BBL.

Patentansprüche

1. Ein offener Fusions-MHC-Ia-Konformer, wobei besagter offener Fusions-MHC-Ia-Konformer ein erstes Monomer oder ein erstes und ein zweites Monomer umfasst oder im Wesentlichen daraus besteht, wobei

a. besagtes erstes Monomer oder jedes von besagtem ersten und zweiten Monomer unabhängig von dem anderen Monomer eine schwere HLA-Kette umfasst, ausgewählt aus C08, A25, B58, A30, B53 und C12, und
b. wobei besagtes erstes Monomer oder jedes von besagtem ersten und zweiten Monomer kovalent mit einer Fc-Polypeptidsequenz verknüpft ist,

wobei die schwere HLA-Kette nur aus den HLA-Alpha-1-, -2 und -3 Domänen besteht.

2. Der offene Fusions-MHC-Ia-Konformer gemäß Anspruch 1, wobei die schwere HLA-Kette C08 ist.

3. Der offene Fusions-MHC-Ia-Konformer gemäß Anspruch 1 oder 2, wobei ein Aminosäure-Linker die schwere HLA-Kette und die Fc-Polypeptidsequenz verbindet.

4. Der offene Fusions-MHC-Ia-Konformer gemäß einem der vorhergehenden Ansprüche, wobei das erste und das zweite Monomer gleich sind.

5. Der offene Fusions-MHC-Ia-Konformer gemäß einem der vorhergehenden Ansprüche, wobei der offene Fusions-MHC-Ia-Konformer zusätzlich ein Peptid-Epitop-Fragment umfasst, insbesondere wobei das erste und/oder zweite Monomer zusätzlich ein Peptid-Epitop-Fragment umfasst.

6. Der offene Fusions-MHC-Ia-Konformer gemäß einem der vorhergehenden Ansprüche, wobei die Fc-Domäne konstante Regionen der schweren Kette C_H2 und C_H3 umfasst, ausgewählt aus einem von Immunglobulin Typ G (IgG), Typ A (IgA), Typ D (IgD), Typ E (IgE) oder Typ M (IgM).

7. Der offene Fusions-MHC-Ia-Konformer gemäß einem der vorhergehenden Ansprüche, wobei der Aminosäure-Linker 1 bis 50, insbesondere 5 bis 40, weiter insbesondere 10 bis 30, noch weiter insbesondere 15 bis 25 Aminosäuren umfasst, die die schwere HLA-Kette mit der Fc-Domäne als eine einzige Polypeptidkette verknüpfen.

8. Der offene Fusions-MHC-Ia-Konformer gemäß einem der vorhergehenden Ansprüche zur Verwendung als Medikament.

9. Der offene Fusions-MHC-Ia-Konformer gemäß einem der vorhergehenden Ansprüche 1 bis 7 zur Verwendung

   a. in der Behandlung oder Prävention von Krebs, oder
   b. als immunmodulatorisches Agens, insbesondere bei der Behandlung einer Infektionskrankheit, insbesondere bei der Behandlung von Infektionen mit dem Humanen Immundefizienz-Virus (HIV), Hepatitis A, Hepatitis B, Hepatitis C, Influenza, Infektionen mit dem Respiratorischen Synzytial-Virus (RSV), Masern, Herpes und Gelbfieber.

10. Ein Nukleinsäuremolekül, wobei besagtes Nukleinsäuremolekül ein offenes Fusions-MHC-Ia-Konformer-Monomer gemäß einem der Ansprüche 1 bis 7 kodiert.

11. Ein Virus, umfassend das Nukleinsäuremolekül nach Anspruch 10 unter Kontrolle einer Promotorsequenz, die in einer Säugetierzelle, insbesondere in einer menschlichen Zelle, funktionsfähig ist, insbesondere ein Adenovirus, ein Adeno-assoziiertes Virus, ein Herpesvirus oder ein Lentivirus.

12. Eine *in-vitro* genetisch modifizierte Wirtszelle, umfassend das Nukleinsäuremolekül nach Anspruch 10.

13. Ein Kombinationsmedikament, umfassend

   a. einen offenen Fusions-MHC-Ia-Konformer wie in einem der Ansprüche 1 bis 7 angegeben, und
   b. ein Checkpoint-modulierendes Agens, ausgewählt aus

      i. ein Checkpoint-inhibitorisches Agens (CPI), insbesondere wobei besagtes CPI ausgewählt ist aus:

         - einem Inhibitor der Wechselwirkung von CTLA4 mit entweder B7-1 (cd80) und/oder B7-2 (cd86), insbesondere einem Polypeptidliganden für CTLA-4 oder für cd80 oder für cd86;
         - einem Inhibitor der Wechselwirkung von PD-1 mit entweder PD-L1 und/oder PD-L2, insbesondere einem Polypeptid ligand für PD-1 oder für PD-L1 oder für PD-L2; und
         - einem inhibitorischen Polypeptidliganden, insbesondere einem Antikörper, von T-Zell-Immunglobulin und Mucin-Domäne enthaltend 3 (TIM-3); und

      ii. einem Checkpoint-agonistischen Agens, insbesondere einem Checkpoint-agonistischen Antikörper, der so ausgewählt ist, dass er an den Tumornekrosefaktor-Rezeptor 4-1BB bindet und diesen aktiviert, insbesondere einem monoklonalen Antikörper gegen 4-1BB;

   insbesondere wobei besagtes Checkpoint-modulierendes Agens ein Polypeptid ist, ausgewählt aus einem Antikörper, einem Antikörperfragment und einem Antikörperähnlichen Molekül, und das Polypeptid selektiv reaktiv zu einem

Checkpoint-Mediator ist, ausgewählt aus CTLA4, PD-1, CD80, CD86, PD-L1, PD-L2, TIM-3, 4-1BB und 4-1 BBL.

Revendications

1. Conformère ouvert CMH-Ia de fusion, dans lequel ledit conformère ouvert CMH-Ia de fusion comprend ou est essentiellement constitué d'un premier monomère ou d'un premier et d'un second monomère, dans lequel

   a. ledit premier monomère, ou chacun desdits premier et second monomères indépendamment de l'autre monomère, comprend une chaîne lourde de HLA sélectionnée parmi C08, A25, B58, A30, B53, et C12, et
   b. dans lequel ledit premier monomère, ou chacun desdits premier et second monomères, est lié par covalence à une séquence polypeptidique Fc,

   dans lequel la chaîne lourde de HLA est uniquement constituée des domaines alpha 1, 2 et 3 de HLA.

2. Conformère ouvert CMH-Ia de fusion selon la revendication 1, dans lequel la chaîne lourde de HLA est C08.

3. Conformère ouvert CMH-Ia de fusion selon la revendication 1 ou 2, dans lequel un liant d'acides aminés relie la chaîne lourde de HLA et la séquence polypeptidique Fc.

4. Conformère ouvert CMH-Ia de fusion selon l'une quelconque des revendications précédentes, dans lequel le premier et le second monomère sont identiques.

5. Conformère ouvert CMH-Ia de fusion selon l'une quelconque des revendications précédentes, dans lequel le conformère ouvert CMH-Ia de fusion comprend en outre un fragment de déterminant antigénique peptidique, notamment dans lequel le premier et/ou second monomère comprend en outre un fragment de déterminant antigénique peptidique.

6. Conformère ouvert CMH-Ia de fusion selon l'une quelconque des revendications précédentes, dans lequel le domaine Fc comprend des régions constantes de chaîne lourde C_H2 et C_H3 sélectionnées parmi l'une quelconque d'immunoglobuline de type G (IgG), type A (IgA), type D (IgD), type E (IgE) ou type M (IgM).

7. Conformère ouvert CMH-Ia de fusion selon l'une quelconque des revendications précédentes, dans lequel le liant d'acides aminés comprend 1 à 50, particulièrement 5 à 40, plus particulièrement 10 à 30, encore plus particulièrement 15 à 25 acides aminés, liant la chaîne lourde de HLA au domaine Fc comme une seule chaîne polypeptidique.

8. Conformère ouvert CMH-Ia de fusion selon l'une quelconque des revendications précédentes destiné à être utilisé en tant que médicament.

9. Conformère ouvert CMH-Ia de fusion selon l'une quelconque des revendications précédentes 1 à 7 destiné à être utilisé

   a. dans le traitement ou la prévention du cancer, ou
   b. en tant qu'agent immunomodulateur, particulièrement dans le traitement d'une maladie infectieuse, plus particulièrement dans le traitement d'une infection par le virus de l'immunodéficience humaine (VIH), de l'hépatite A, l'hépatite B, l'hépatite C, de la grippe, d'une infection par le virus respiratoire syncytial (VRS), de la rougeole, de l'herpès et de la fièvre jaune.

10. Molécule d'acide nucléique, dans laquelle ladite molécule d'acide nucléique code pour un monomère conformère ouvert CMH-Ia de fusion selon l'une quelconque des revendications 1 à 7.

11. Virus comprenant la molécule d'acide nucléique selon la revendication 10 sous le contrôle d'une séquence promotrice pouvant être opérée dans une cellule mammifère, notamment dans une cellule humaine, notamment un adénovirus, un virus adéno-associé, un herpèsvirus ou un lentivirus.

12. Cellule hôte génétiquement modifiée *in vitro* comprenant la molécule d'acide nucléique selon la revendication 10.

13. Médicament combiné comprenant

a. un conformère ouvert CMH-Ia de fusion selon l'une quelconque des revendications 1 à 7, et
b. un agent modulateur de checkpoint sélectionné parmi

i. un agent inhibiteur de checkpoint (CPI), particulièrement dans lequel ledit CPI est sélectionné parmi :

- un inhibiteur de l'interaction de CTLA4 avec B7-1 (cd80) et/ou B7-2 (cd86), plus particulièrement un ligand polypeptidique à CTLA-4 ou à cd80 ou à cd86 ;
- un inhibiteur de l'interaction de PD-1 avec PD-L1 et/ou PD-L2, plus particulièrement un ligand polypeptidique à PD-1 ou à PD-L1 ou à PD-L2 ; et
- un ligand polypeptidique inhibiteur, notamment un anticorps, de la protéine-3 des lymphocytes T contenant un domaine d'immunoglobuline et de mucine (TIM-3) ; et

ii. un agent agoniste de checkpoint, notamment un anticorps agoniste de checkpoint sélectionné pour se fixer à et activer le récepteur 4-1BB du facteur de nécrose tumorale, notamment un anticorps monoclonal contre 4-1BB,

notamment dans lequel ledit agent modulateur de checkpoint est un polypeptide sélectionné parmi un anticorps, un fragment d'anticorps, et une molécule de type anticorps, et le polypeptide est sélectivement réactif à un médiateur de checkpoint sélectionné parmi CTLA4, PD-1, CD80, CD86, PD-L1, PD-L2, TIM-3, 4-1BB et 4-1BBL.

Figure 1:

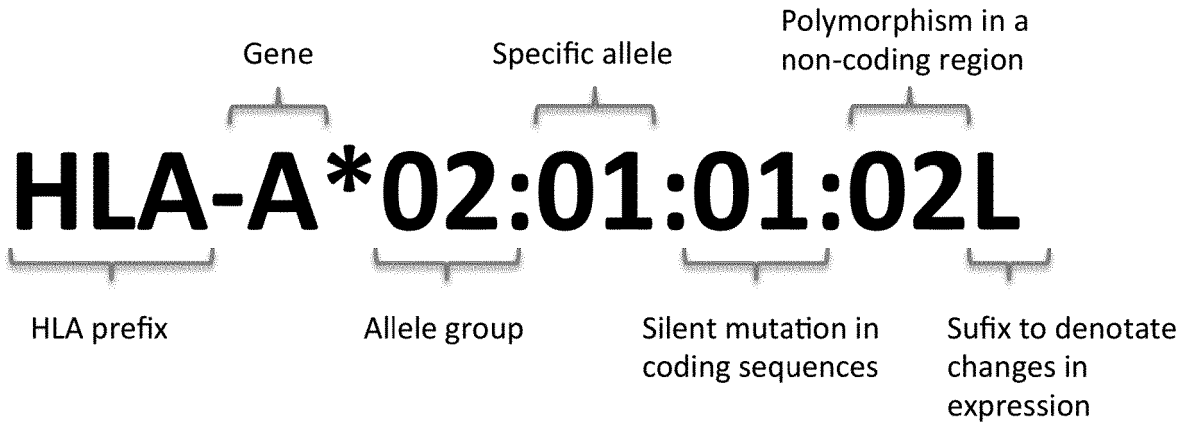

Gene
Specific allele
Polymorphism in a non-coding region
HLA-A*02:01:01:02L
HLA prefix
Allele group
Silent mutation in coding sequences
Sufix to denotate changes in expression

Figure 2:

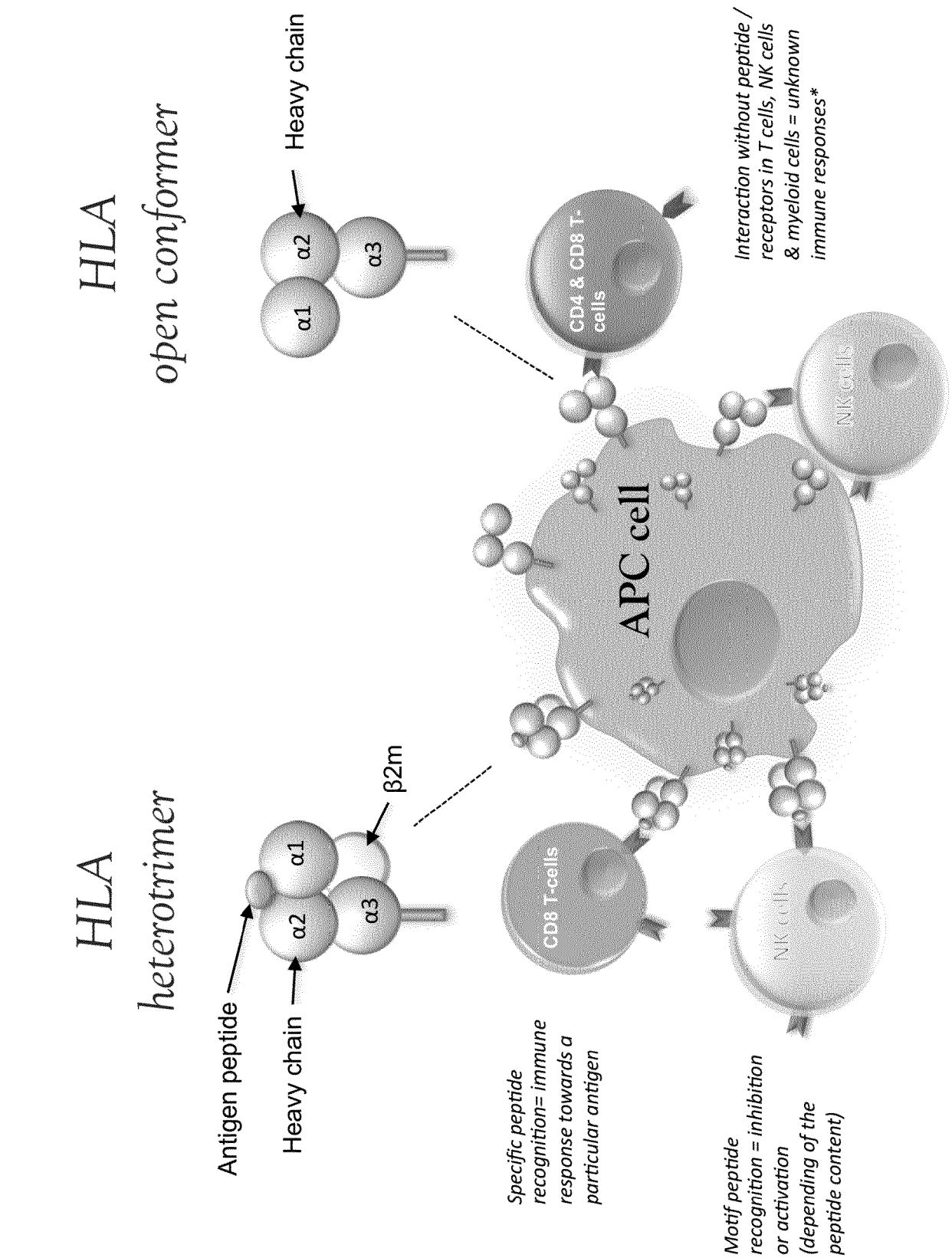
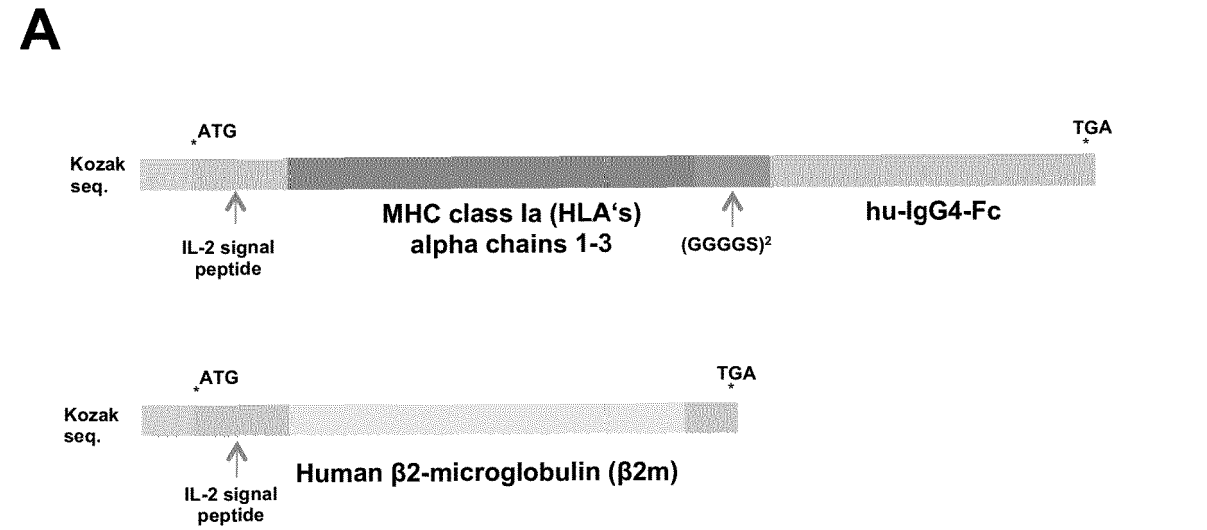

HLA
heterotrimer
HLA
open conformer
Antigen peptide
Heavy chain
α1
α2
α3
β2m
Heavy chain
α1
α2
α3
APC cell
CD8 T-cells
CD4 & CD8 T-cells
NK cells
NK cells
Specific peptide recognition= immune response towards a particular antigen
Motif peptide recognition = inhibition or activation (depending of the peptide content)
Interaction without peptide / receptors in T cells, NK cells & myeloid cells = unknown immune responses*

Figure 3:


A
ATG
TGA
Kozak seq.
IL-2 signal peptide
MHC class Ia (HLA's) alpha chains 1-3
(GGGGS)2
hu-IgG4-Fc
ATG
TGA
Kozak seq.
IL-2 signal peptide
Human β2-microglobulin (β2m)

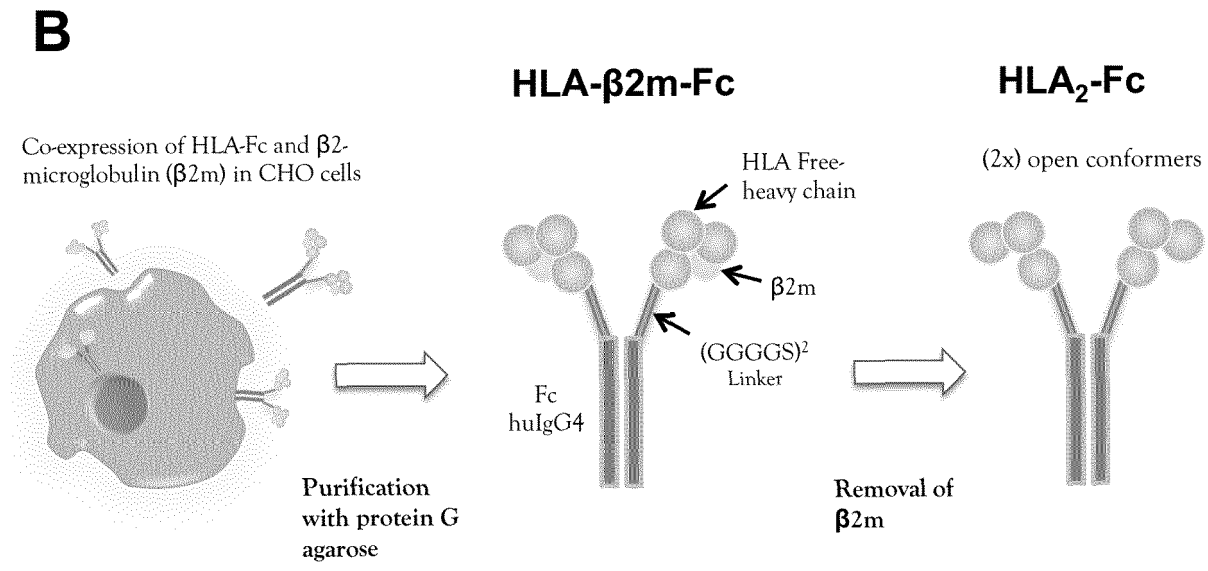

B
HLA-β2m-Fc
HLA2-Fc
Co-expression of HLA-Fc and β2-microglobulin (β2m) in CHO cells
HLA Free-heavy chain
β2m
(GGGGS)2 Linker
Fc huIgG4
(2x) open conformers
Purification with protein G agarose
Removal of β2m

Figure 4:

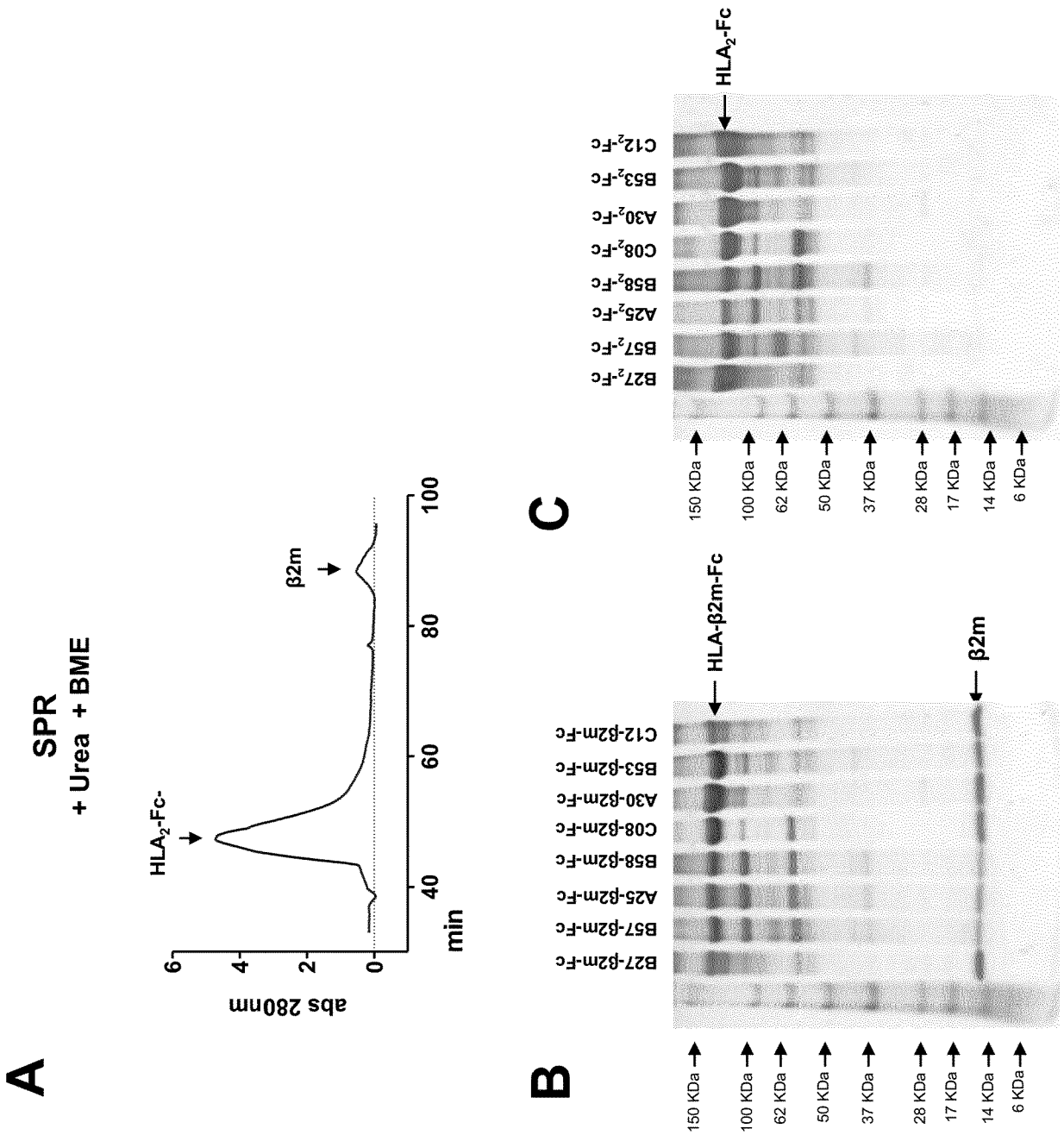

A
SPR
+ Urea + BME
HLA2-Fc-
β2m
abs 280nm
0
2
4
6
40
60
80
100
min
B
HLA-β2m-Fc
β2m
B27-β2m-Fc
B57-β2m-Fc
A25-β2m-Fc
B58-β2m-Fc
C08-β2m-Fc
A30-β2m-Fc
B53-β2m-Fc
C12-β2m-Fc
150 KDa
100 KDa
62 KDa
50 KDa
37 KDa
28 KDa
17 KDa
14 KDa
6 KDa
C
HLA2-Fc
B27z-Fc
B57z-Fc
A25z-Fc
B58z-Fc
C08z-Fc
A30z-Fc
B53z-Fc
C12z-Fc
150 KDa
100 KDa
62 KDa
50 KDa
37 KDa
28 KDa
17 KDa
14 KDa
6 KDa

Figure 5:

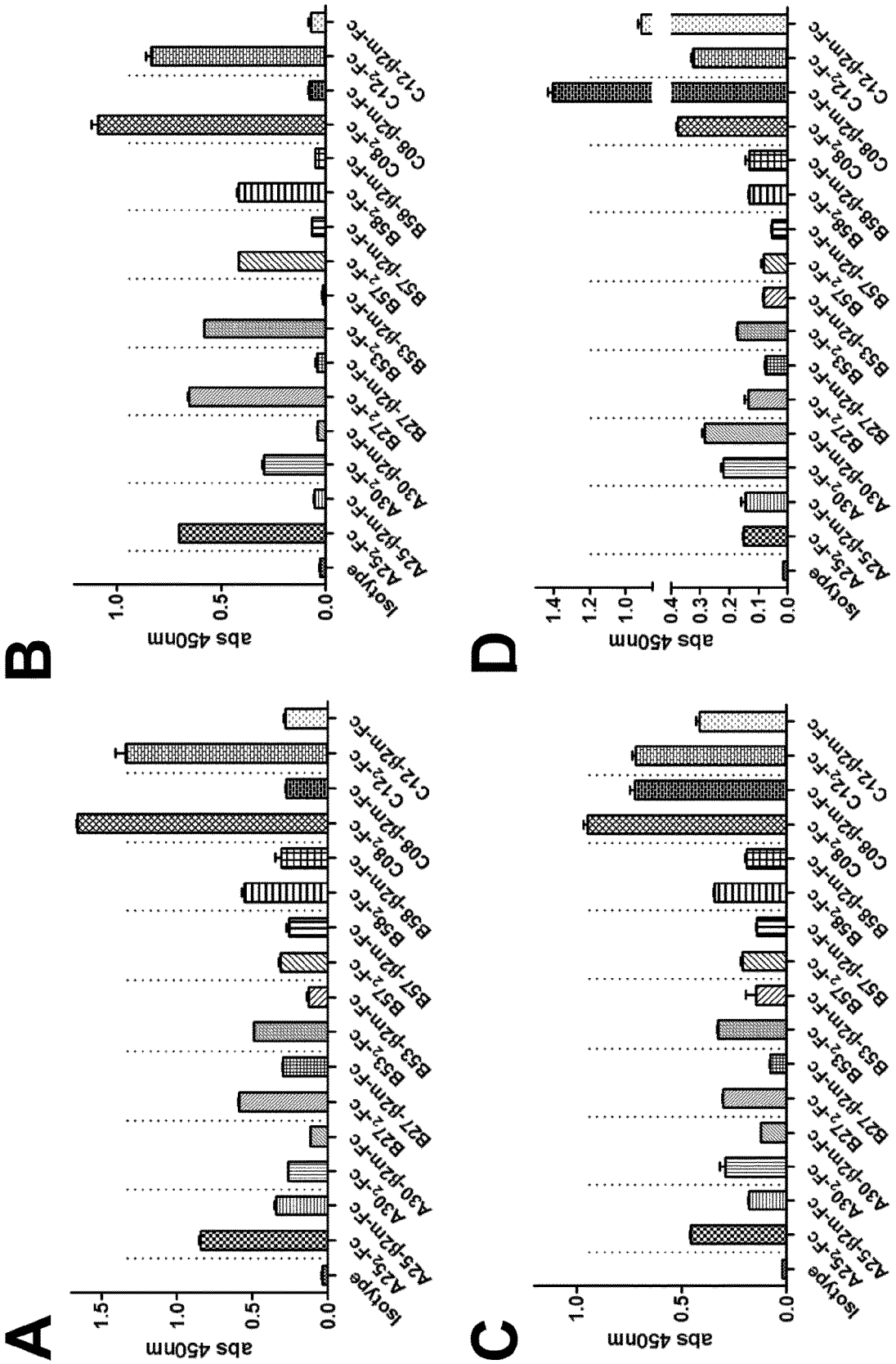

A
B
C
D
abs 450nm
isotype
A25-fc
A25-β2m-fc
A30-fc
A30-β2m-fc
B27-fc
B27-β2m-fc
B53-fc
B53-β2m-fc
B57-fc
B57-β2m-fc
B58-fc
B58-β2m-fc
C08-fc
C08-β2m-fc
C12-fc
C12-β2m-fc

Figure 5 (continued):

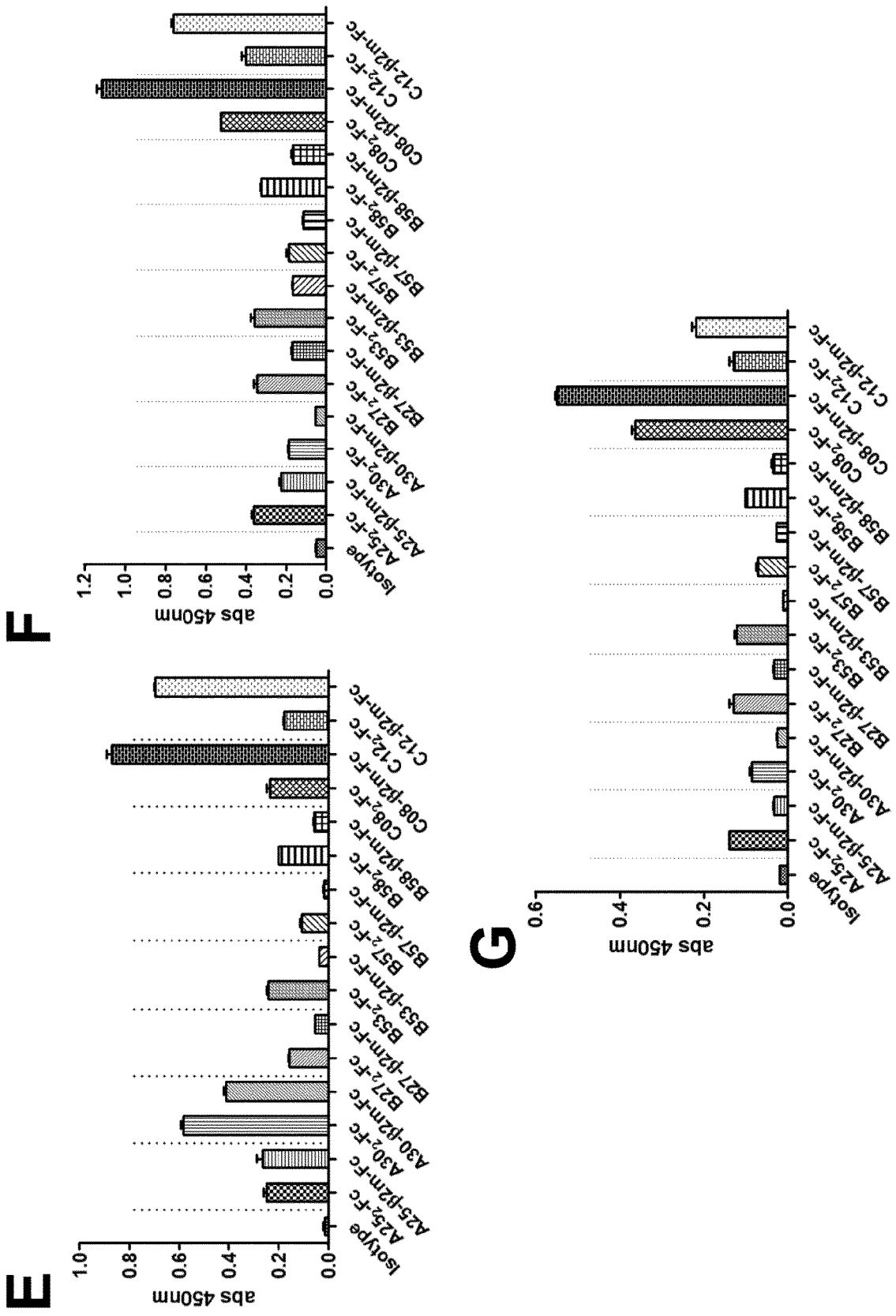


Figure 6:

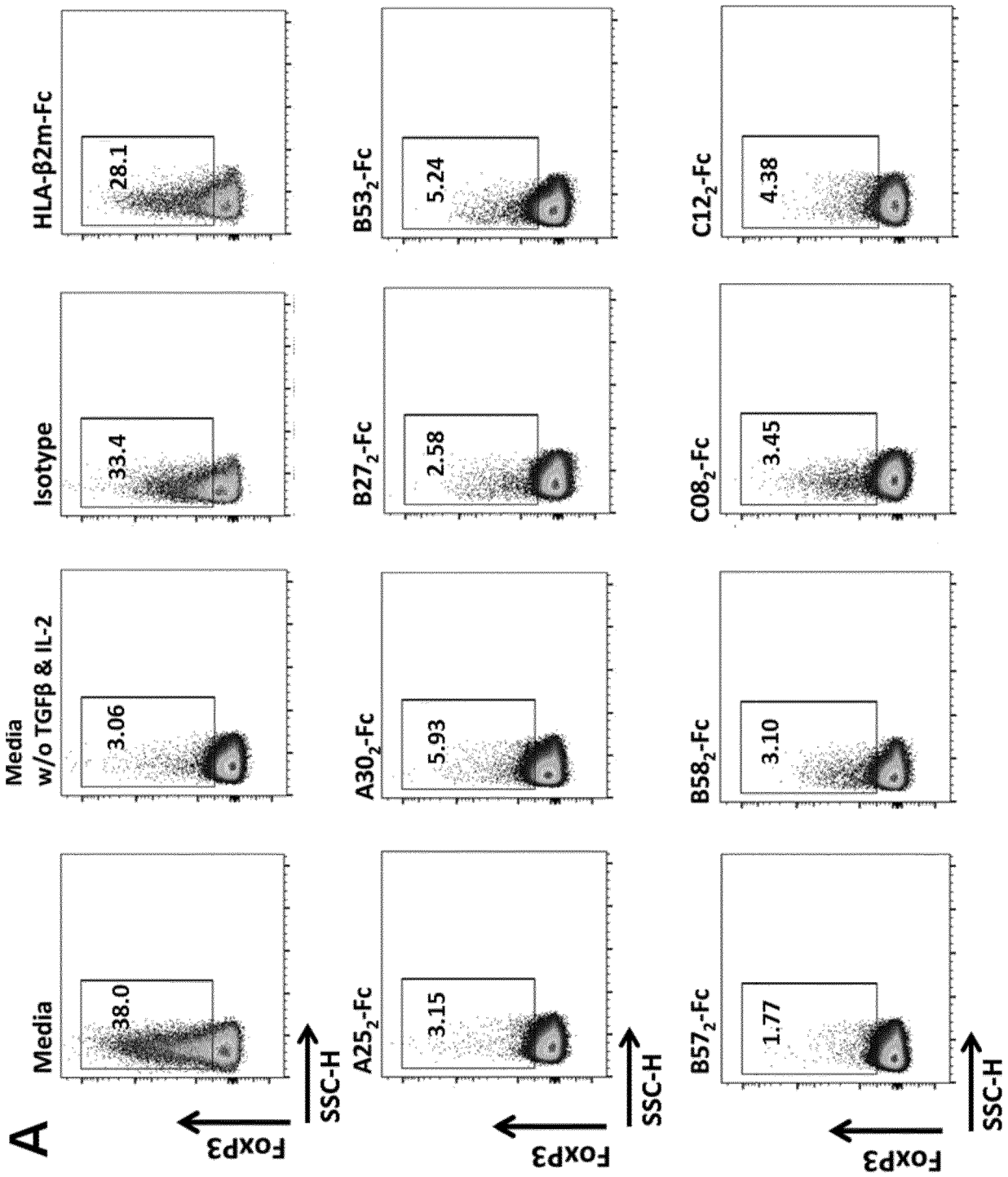

A
Media
38.0
Media w/o TGFβ & IL-2
3.06
Isotype
33.4
HLA-β2m-Fc
28.1
A25$_2$-Fc
3.15
A30$_2$-Fc
5.93
B27$_2$-Fc
2.58
B53$_2$-Fc
5.24
B57$_2$-Fc
1.77
B58$_2$-Fc
3.10
C08$_2$-Fc
3.45
C12$_2$-Fc
4.38
FoxP3
SSC-H

Figure 6 (continued):

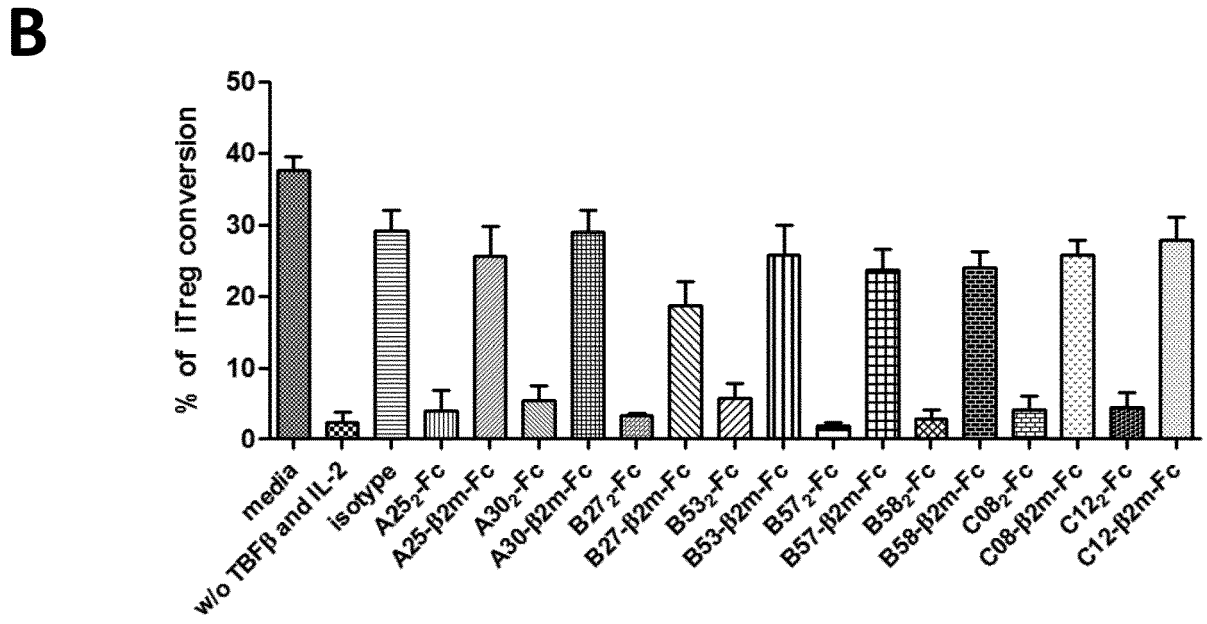


Figure 7:

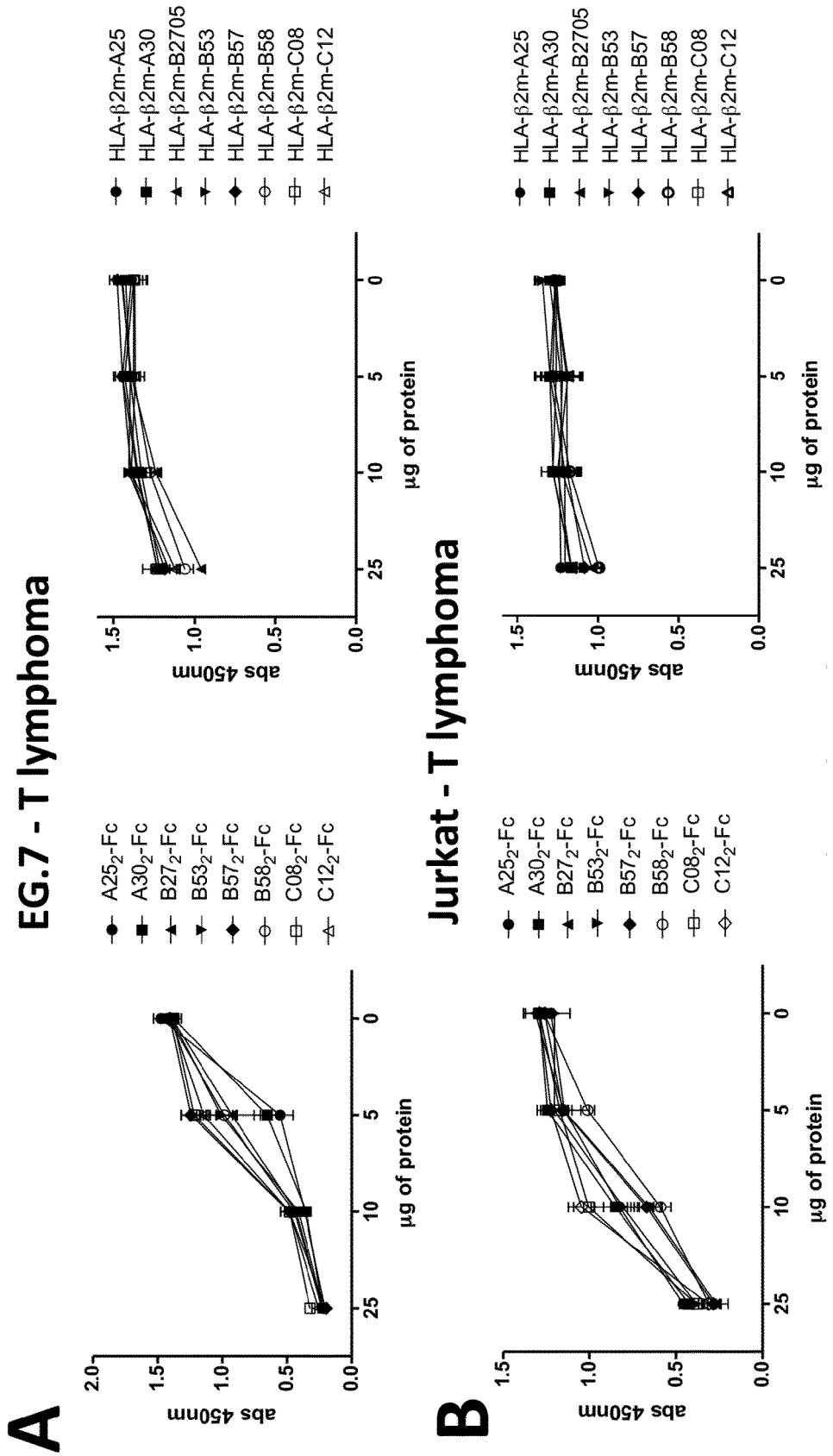

A
EG.7 - T lymphoma
A25$_2$-Fc
A30$_2$-Fc
B27$_2$-Fc
B53$_2$-Fc
B57$_2$-Fc
B58$_2$-Fc
C08$_2$-Fc
C12$_2$-Fc
abs 450nm
µg of protein
HLA-β2m-A25
HLA-β2m-A30
HLA-β2m-B2705
HLA-β2m-B53
HLA-β2m-B57
HLA-β2m-B58
HLA-β2m-C08
HLA-β2m-C12
B
Jurkat - T lymphoma

Figure 7 (continued):

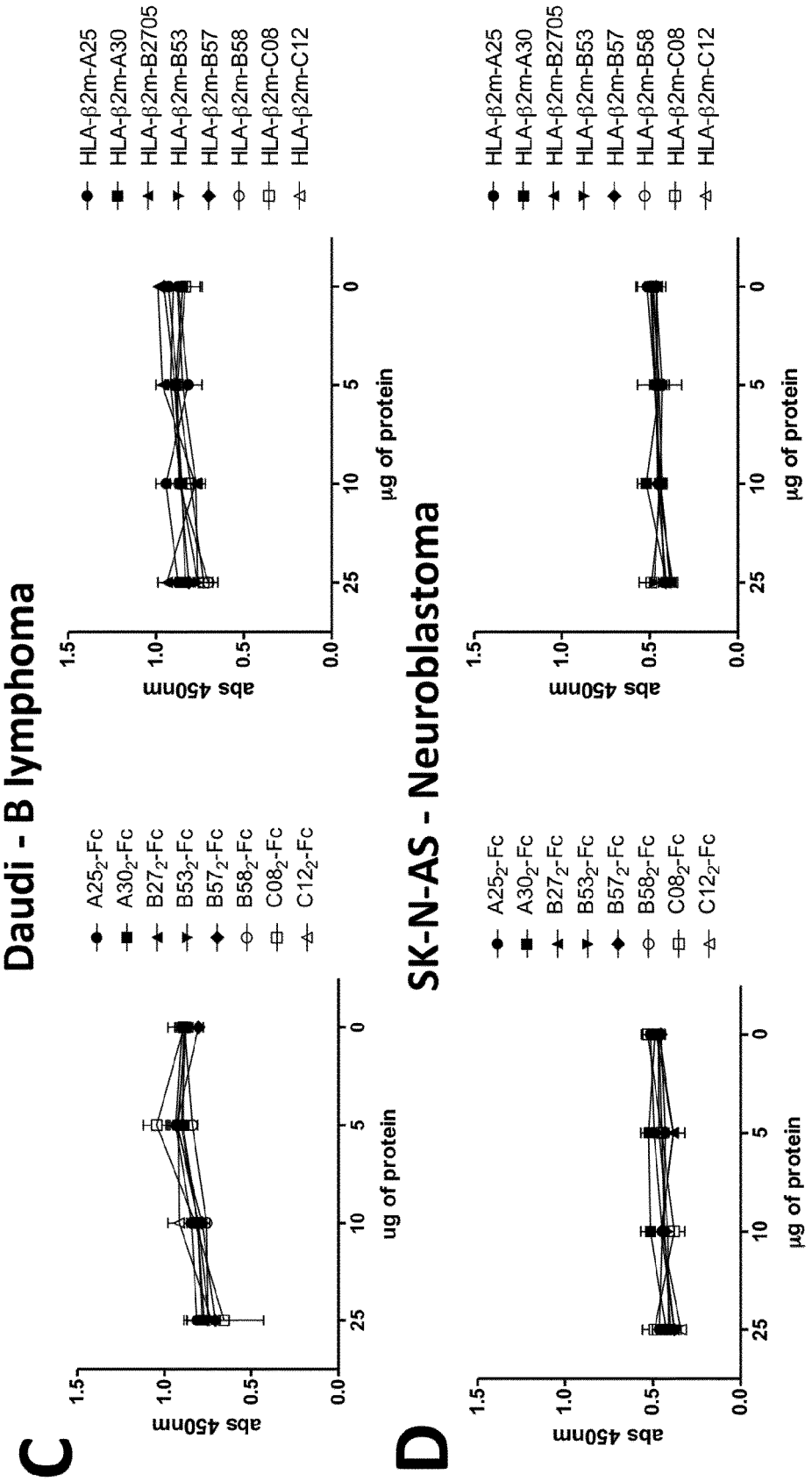


Figure 7 (continued):

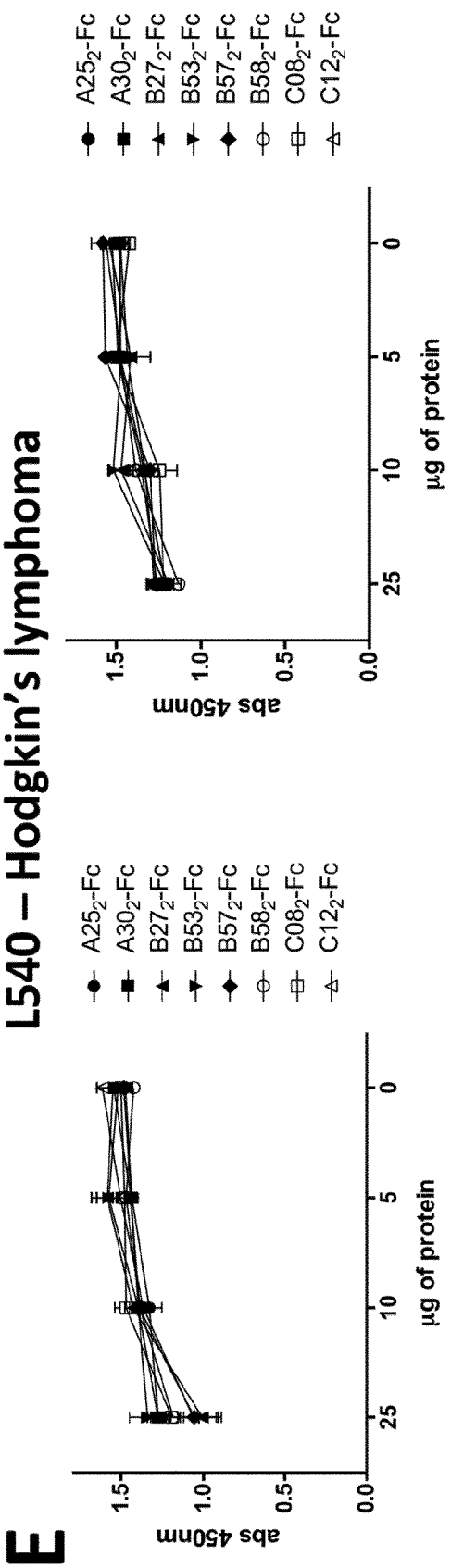
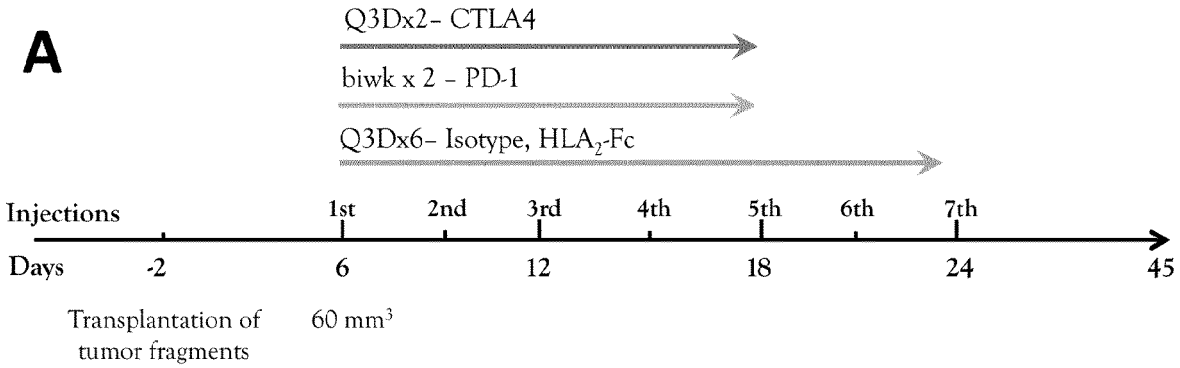
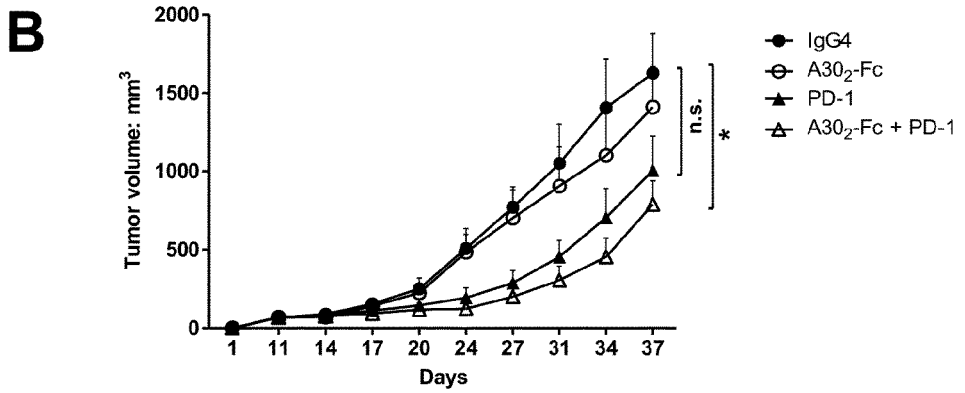
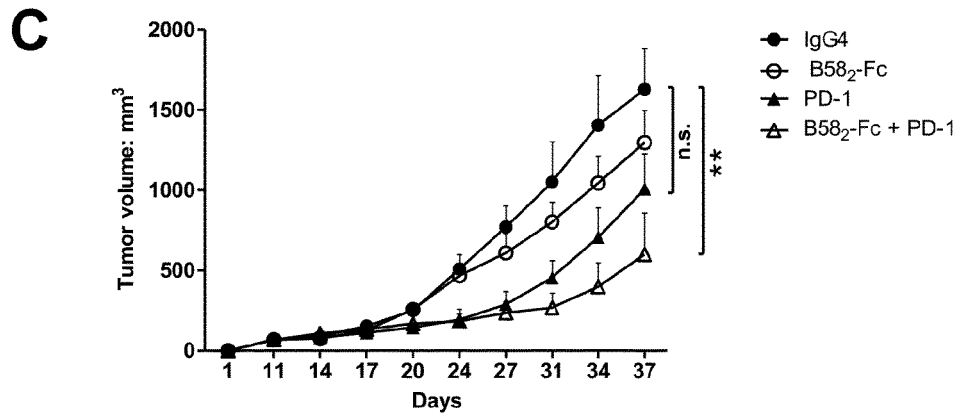


Figure 8:


A
Q3Dx2- CTLA4
biwk x 2 - PD-1
Q3Dx6- Isotype, HLA2-Fc
Injections
1st
2nd
3rd
4th
5th
6th
7th
Days
-2
6
12
18
24
45
Transplantation of tumor fragments
60 mm3


B
Tumor volume: mm3
2000
1500
1000
500
0
1
11
14
17
20
24
27
31
34
37
Days
n.s.
*
IgG4
A302-Fc
PD-1
A302-Fc + PD-1


C
Tumor volume: mm3
2000
1500
1000
500
0
1
11
14
17
20
24
27
31
34
37
Days
n.s.
**
IgG4
B582-Fc
PD-1
B582-Fc + PD-1

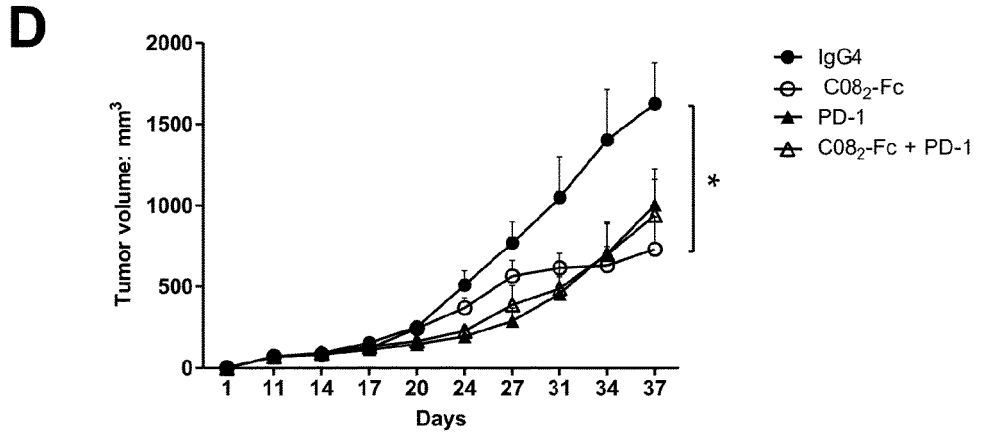

D
Tumor volume: mm3
2000
1500
1000
500
0
1
11
14
17
20
24
27
31
34
37
Days
*
IgG4
C082-Fc
PD-1
C082-Fc + PD-1

Figure 9:

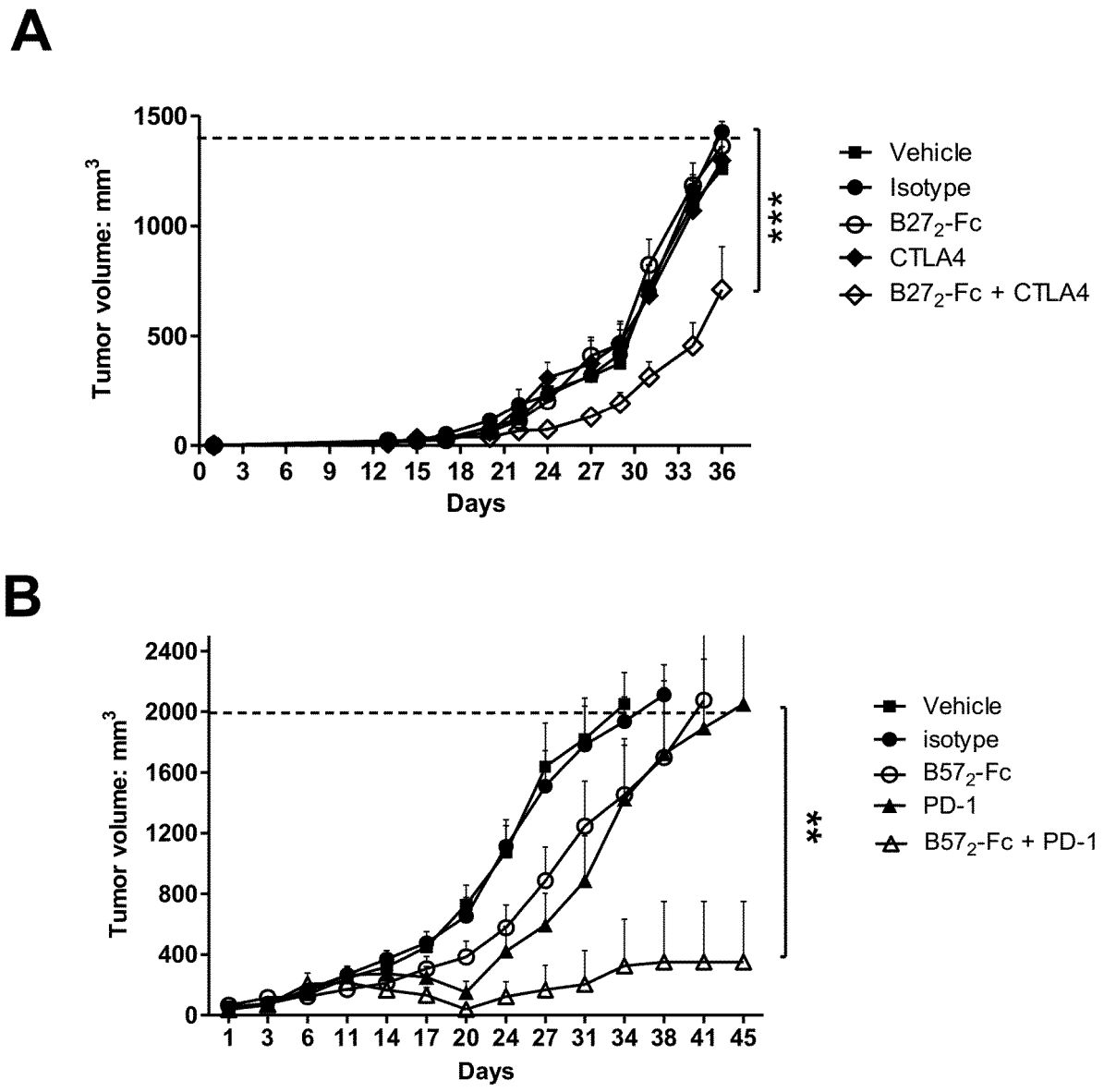

A
1500
1000
500
0
Tumor volume: mm3
0 3 6 9 12 15 18 21 24 27 30 33 36
Days
***
Vehicle
Isotype
B27$_2$-Fc
CTLA4
B27$_2$-Fc + CTLA4
B
2400
2000
1600
1200
800
400
0
Tumor volume: mm3
1 3 6 11 14 17 20 24 27 31 34 38 41 45
Days
**
Vehicle
isotype
B57$_2$-Fc
PD-1
B57$_2$-Fc + PD-1

Figure 10:

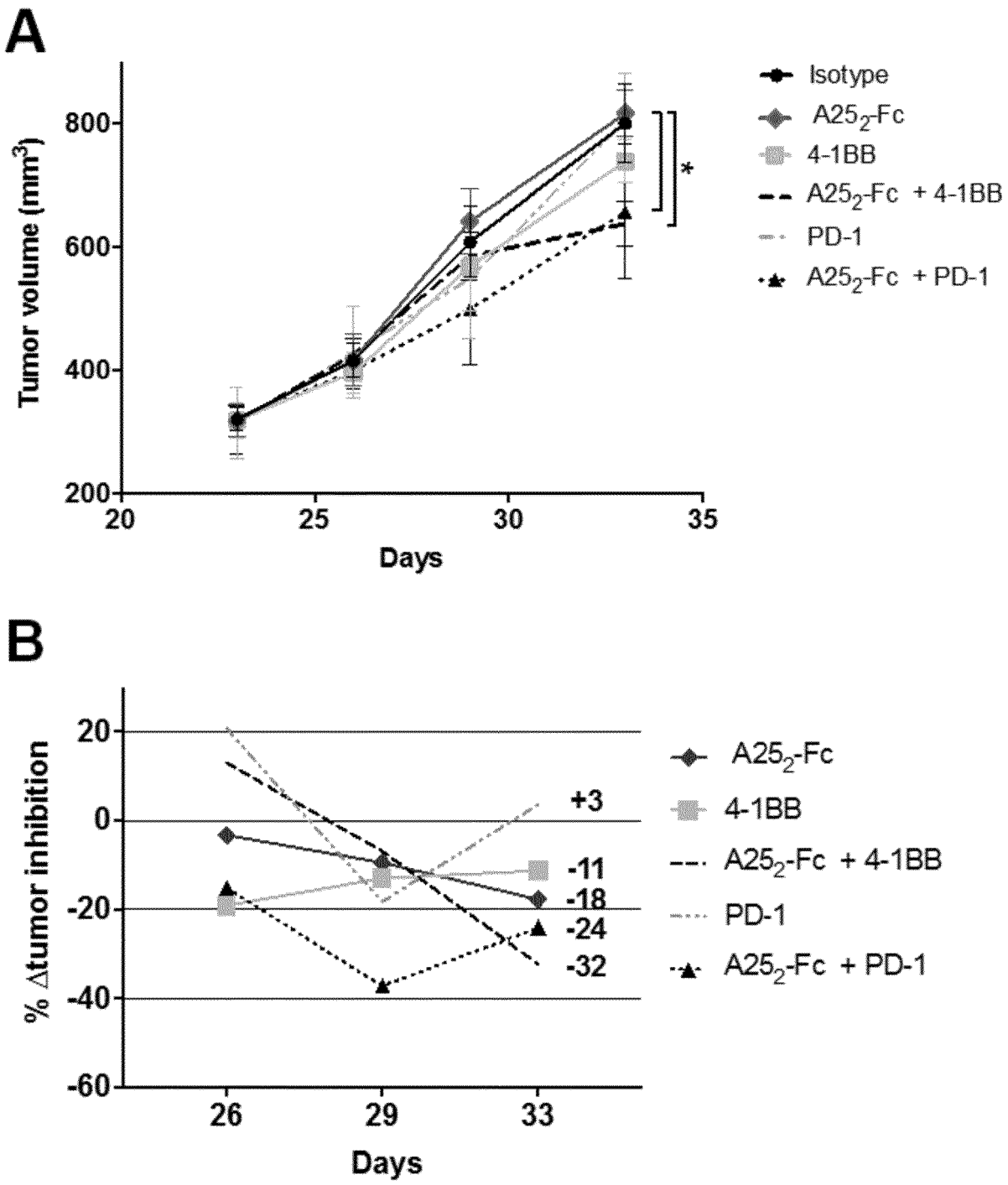

A
Tumor volume (mm3)
800
600
400
200
20
25
30
35
Days
*
Isotype
A252-Fc
4-1BB
A252-Fc + 4-1BB
PD-1
A252-Fc + PD-1
B
% Δtumor inhibition
20
0
-20
-40
-60
26
29
33
Days
+3
-11
-18
-24
-32
A252-Fc
4-1BB
A252-Fc + 4-1BB
PD-1
A252-Fc + PD-1

Figure 11:

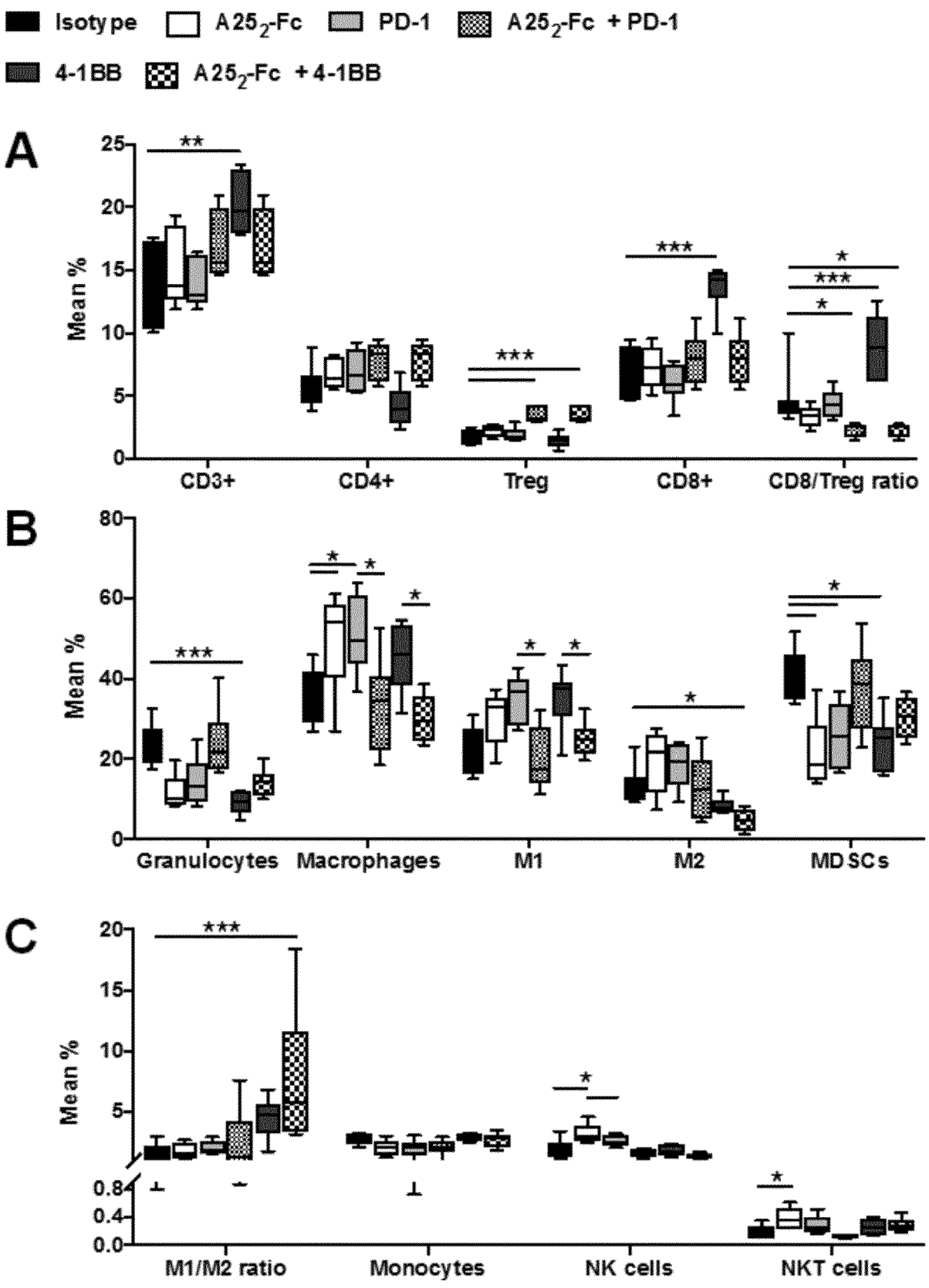

Isotype
A25$_2$-Fc
PD-1
A25$_2$-Fc + PD-1
4-1BB
A25$_2$-Fc +4-1BB
A
Mean %
CD3+
CD4+
Treg
CD8+
CD8/Treg ratio
B
Granulocytes
Macrophages
M1
M2
MDSCs
C
M1/M2 ratio
Monocytes
NK cells
NKT cells

Figure 12:

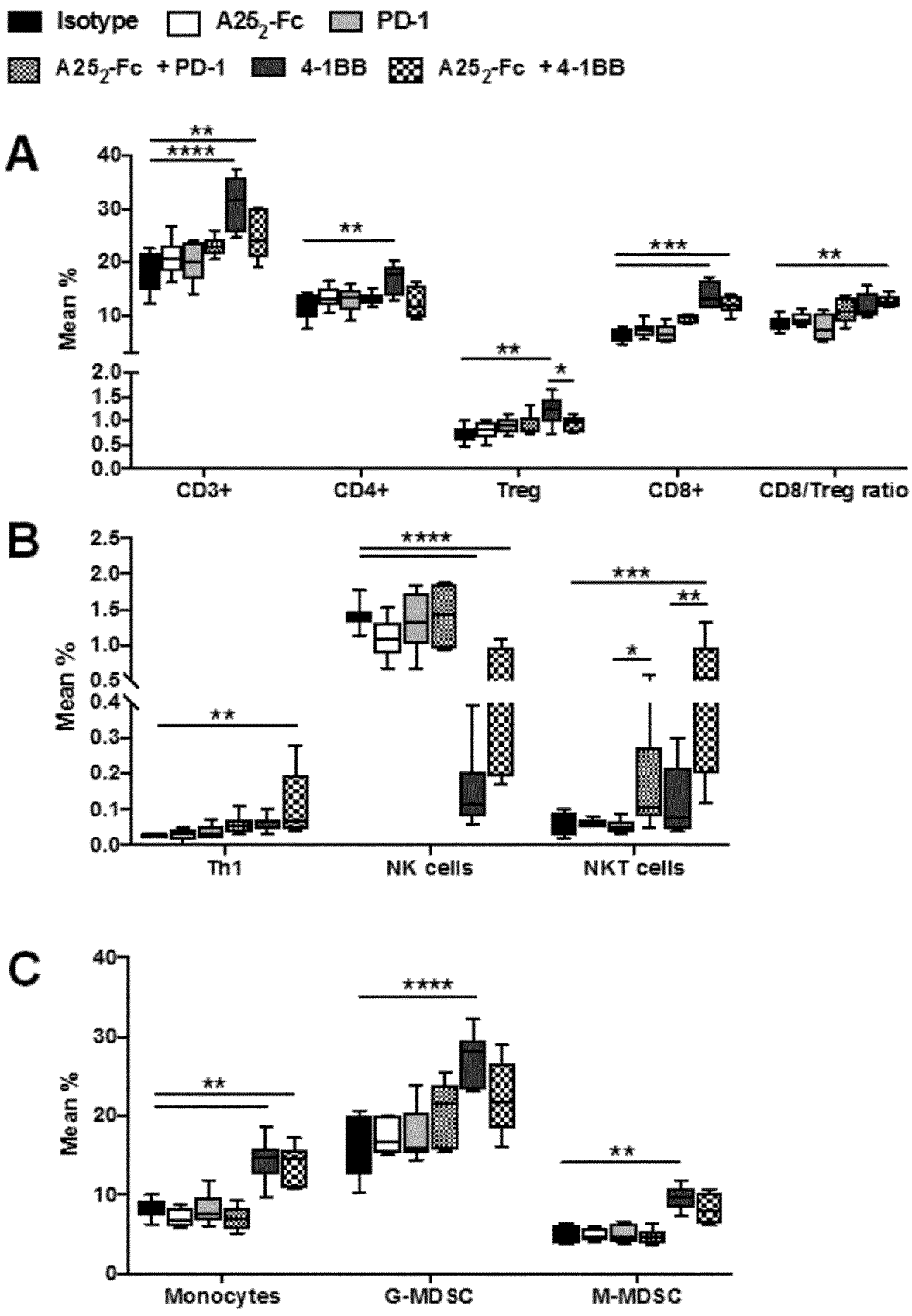

Isotype
A25$_2$-Fc
PD-1
A25$_2$-Fc +PD-1
4-1BB
A25$_2$-Fc +4-1BB
A
Mean %
CD3+
CD4+
Treg
CD8+
CD8/Treg ratio
B
Mean %
Th1
NK cells
NKT cells
C
Mean %
Monocytes
G-MDSC
M-MDSC

Figure 13:

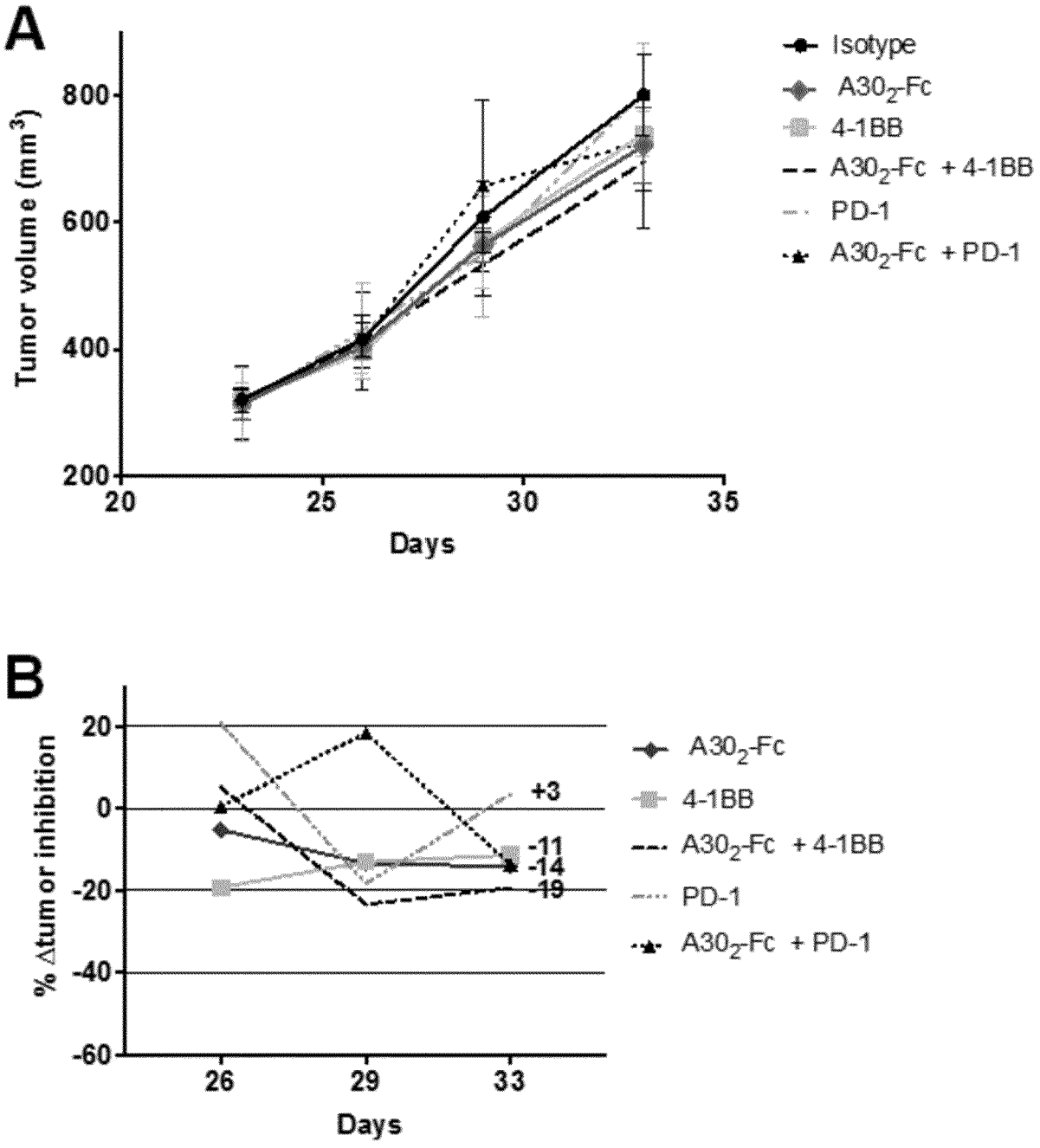

A
Tumor volume (mm3)
800
600
400
200
20
25
30
35
Days
Isotype
A302-Fc
4-1BB
A302-Fc + 4-1BB
PD-1
A302-Fc + PD-1
B
% Δtumor inhibition
20
0
-20
-40
-60
26
29
33
Days
+3
-11
-14
-19
A302-Fc
4-1BB
A302-Fc + 4-1BB
PD-1
A302-Fc + PD-1

Figure 14:

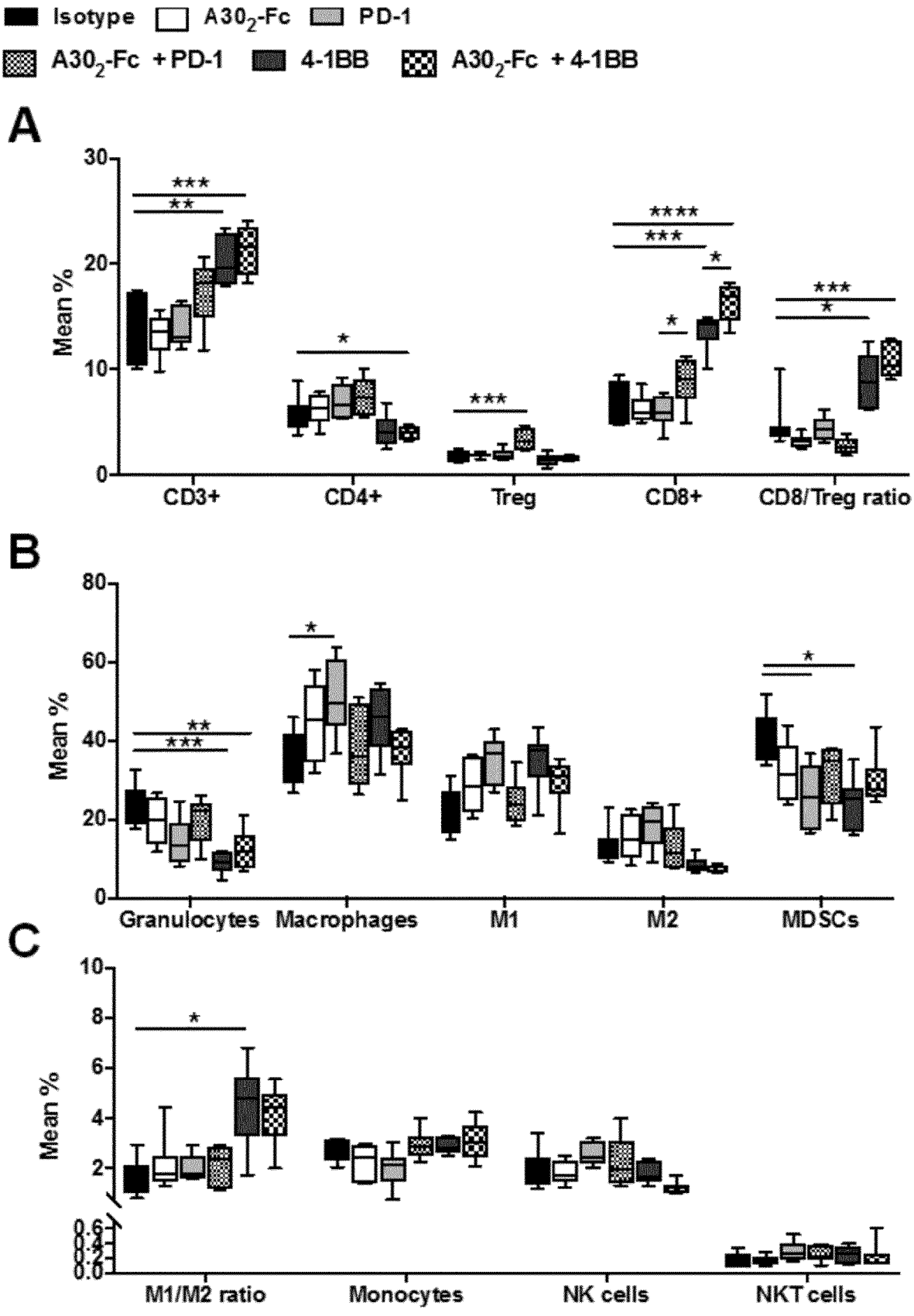

Isotype
A30$_2$-Fc
PD-1
A30$_2$-Fc +PD-1
4-1BB
A30$_2$-Fc +4-1BB
A
Mean %
CD3+
CD4+
Treg
CD8+
CD8/Treg ratio
B
Granulocytes
Macrophages
M1
M2
MDSCs
C
M1/M2 ratio
Monocytes
NK cells
NKT cells

Figure 15:

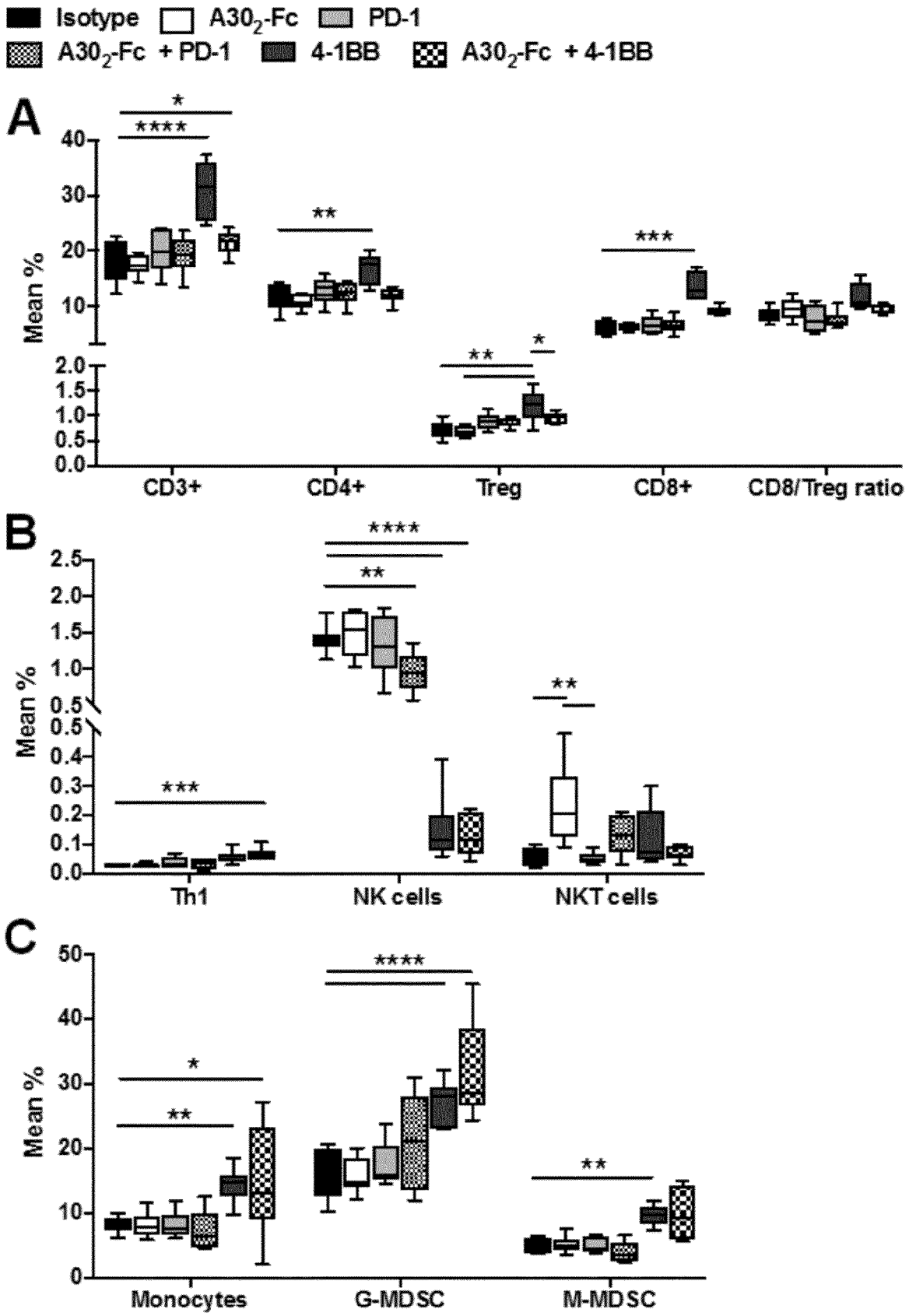

Isotype
A30$_2$-Fc
PD-1
A30$_2$-Fc + PD-1
4-1BB
A30$_2$-Fc + 4-1BB
A
Mean %
CD3+
CD4+
Treg
CD8+
CD8/Treg ratio
B
Mean %
Th1
NK cells
NKT cells
C
Mean %
Monocytes
G-MDSC
M-MDSC

Figure 16:

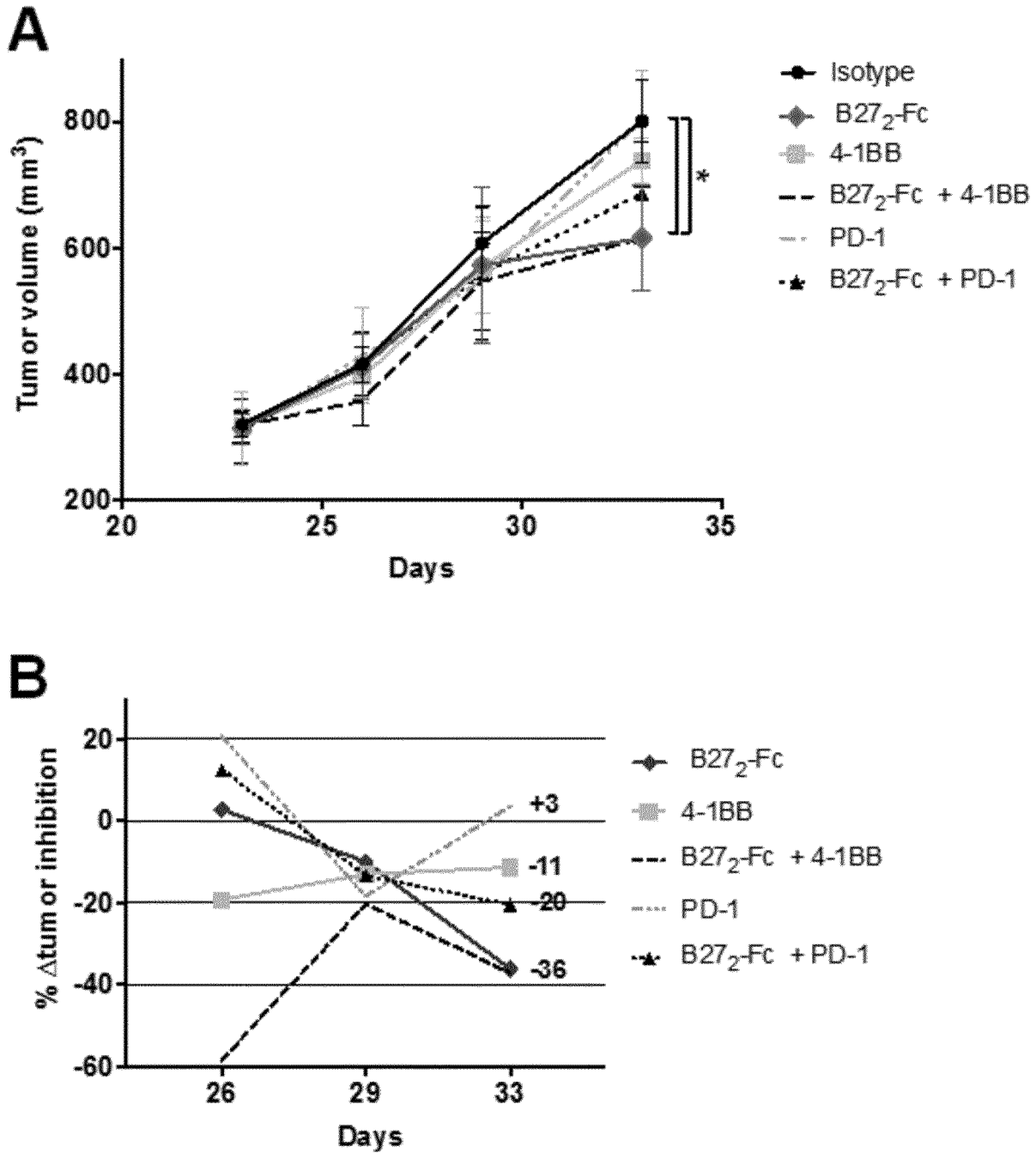

A
Tumor volume (mm3)
800
600
400
200
20
25
30
35
Days
*
Isotype
B27$_2$-Fc
4-1BB
B27$_2$-Fc + 4-1BB
PD-1
B27$_2$-Fc + PD-1
B
% Δtumor inhibition
20
0
-20
-40
-60
26
29
33
Days
+3
-11
-20
-36
B27$_2$-Fc
4-1BB
B27$_2$-Fc + 4-1BB
PD-1
B27$_2$-Fc + PD-1

Figure 17:

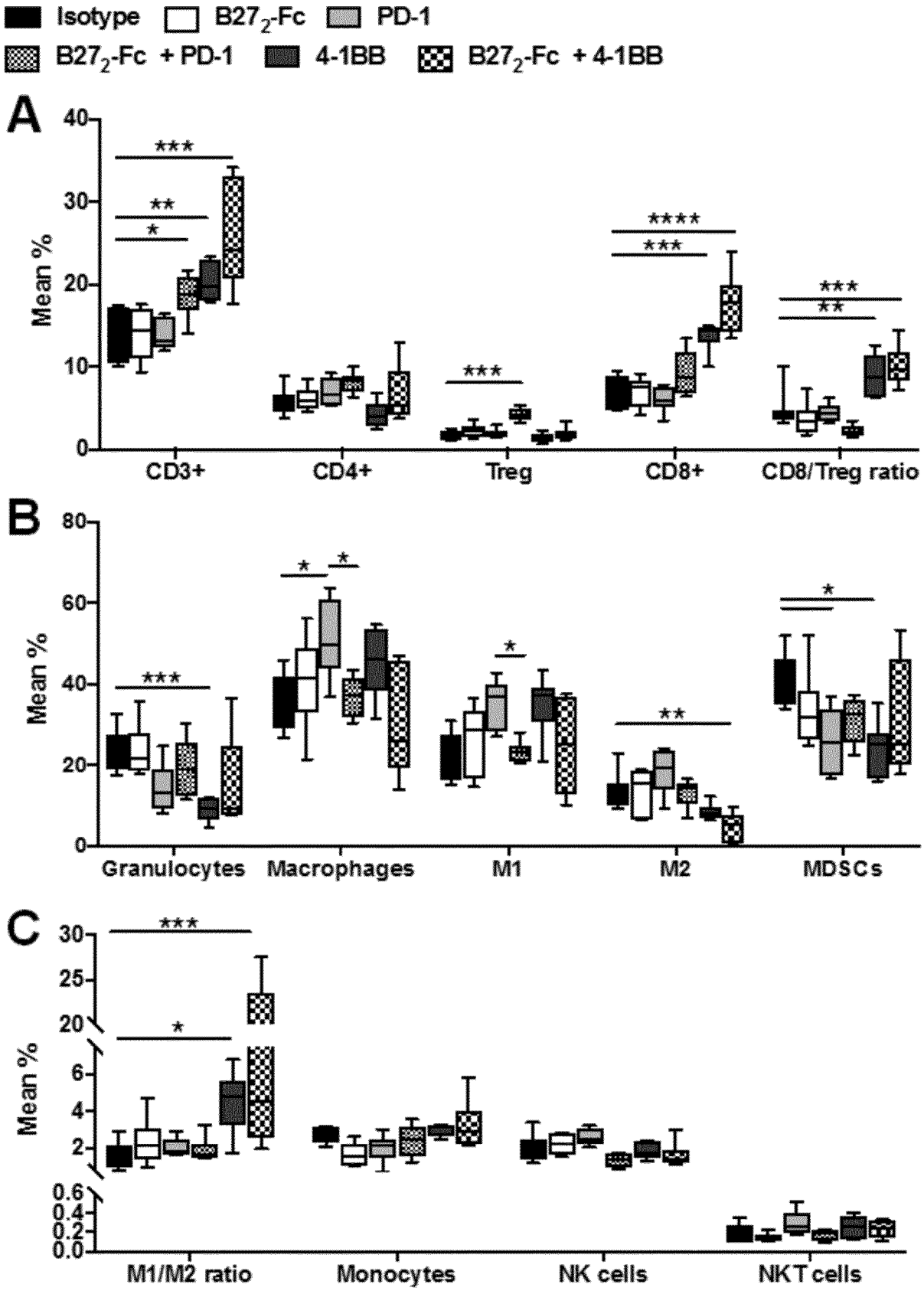

Isotype
B27$_2$-Fc
PD-1
B27$_2$-Fc + PD-1
4-1BB
B27$_2$-Fc + 4-1BB
A
Mean %
CD3+
CD4+
Treg
CD8+
CD8/Treg ratio
B
Granulocytes
Macrophages
M1
M2
MDSCs
C
M1/M2 ratio
Monocytes
NK cells
NKT cells

Figure 18:

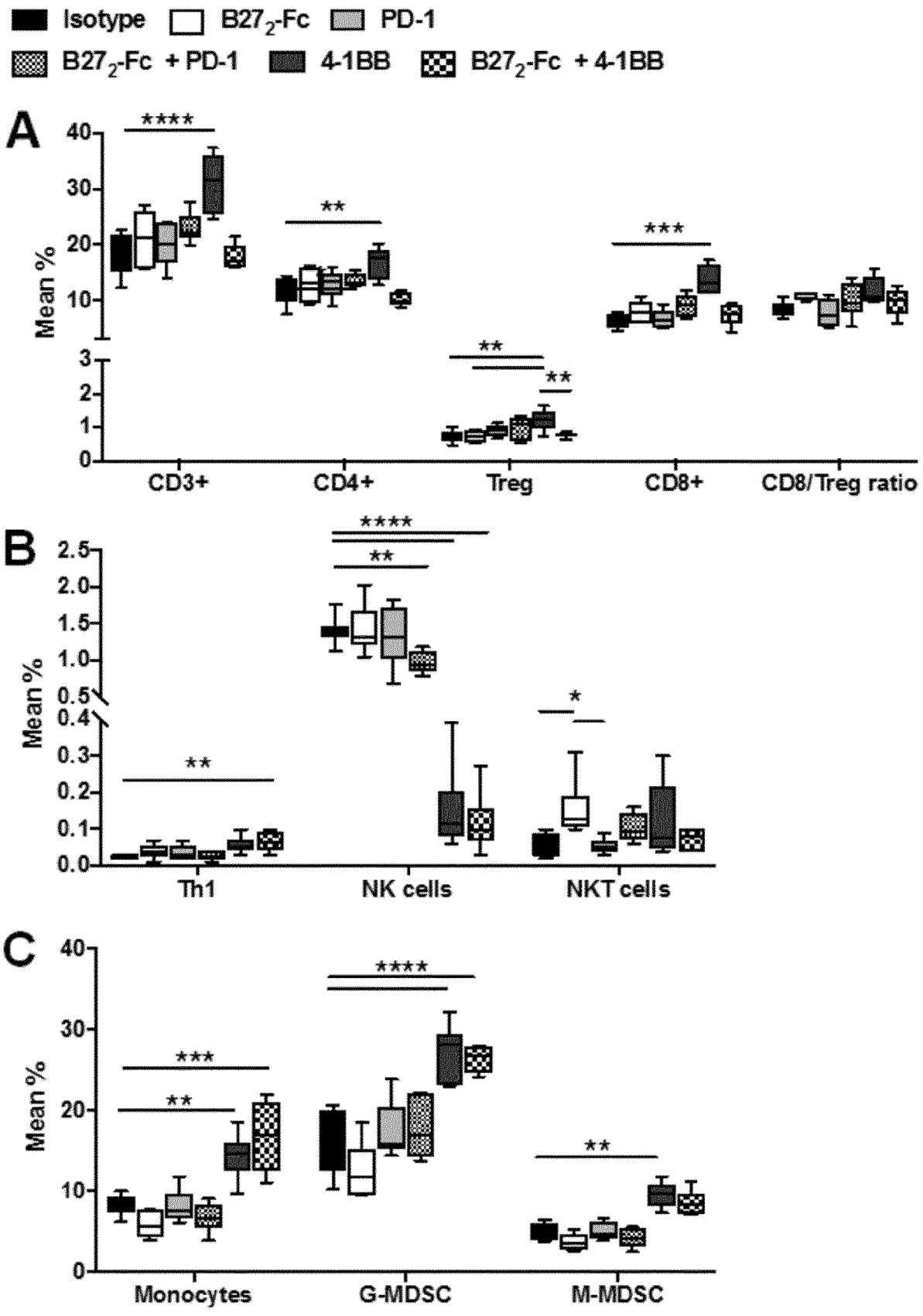

Isotype
B27$_2$-Fc
PD-1
B27$_2$-Fc + PD-1
4-1BB
B27$_2$-Fc + 4-1BB
A
Mean %
CD3+
CD4+
Treg
CD8+
CD8/Treg ratio
B
Th1
NK cells
NKT cells
C
Monocytes
G-MDSC
M-MDSC

Figure 19:

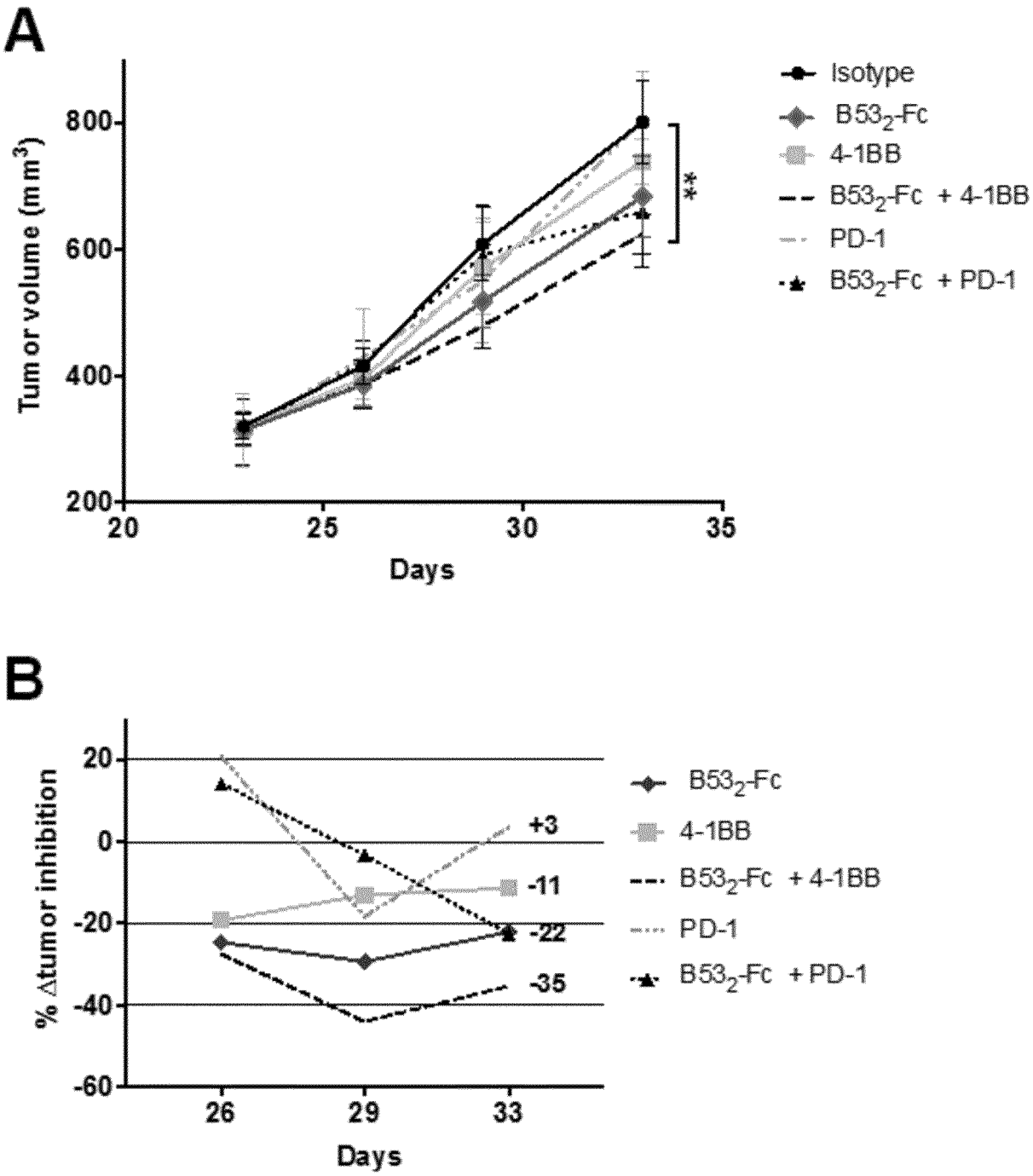

A
Tumor volume (mm3)
800
600
400
200
20
25
30
35
Days
**
Isotype
B532-Fc
4-1BB
B532-Fc + 4-1BB
PD-1
B532-Fc + PD-1
B
% Δtumor inhibition
20
0
-20
-40
-60
26
29
33
Days
+3
-11
-22
-35
B532-Fc
4-1BB
B532-Fc + 4-1BB
PD-1
B532-Fc + PD-1

Figure 20:

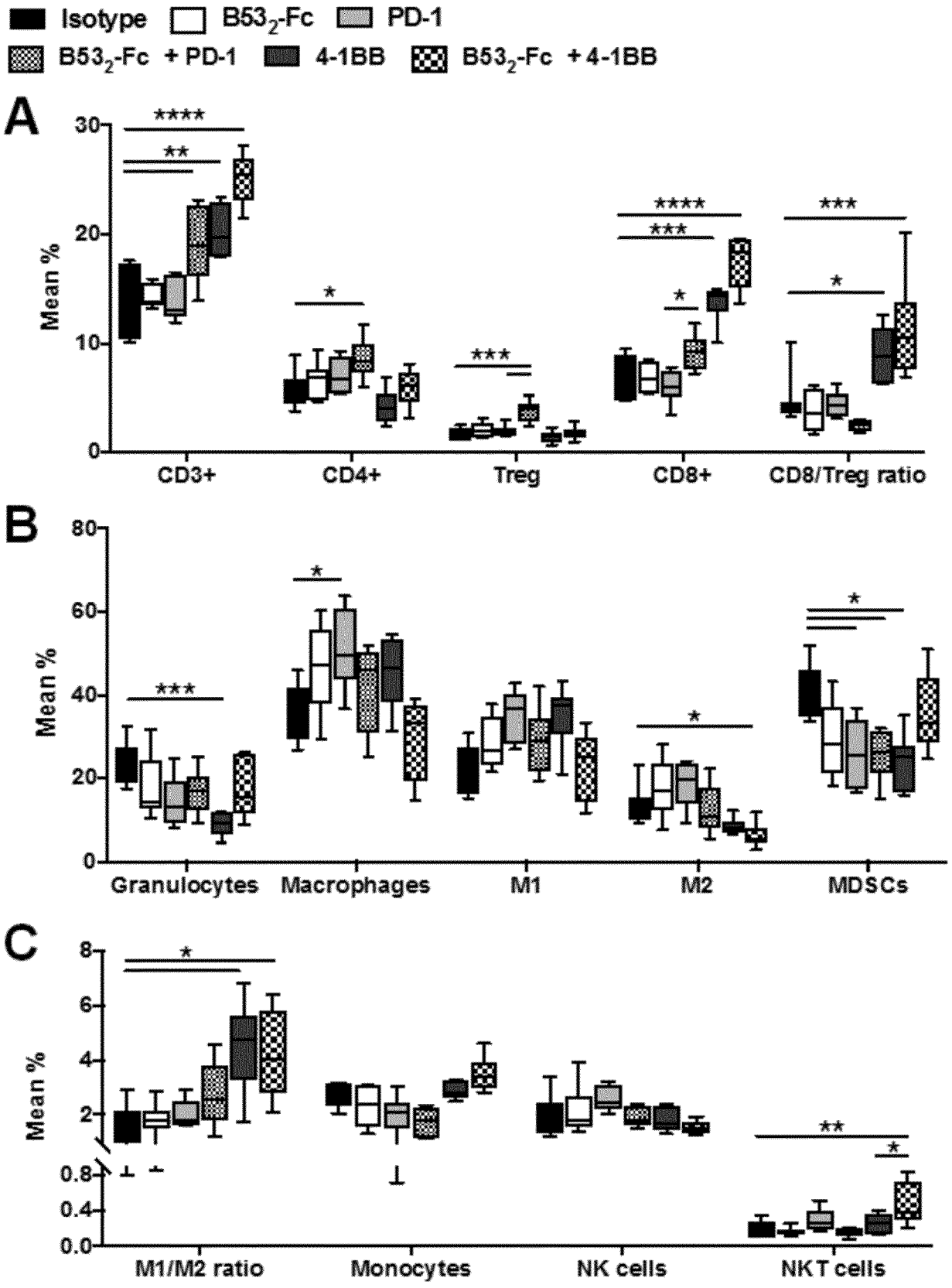

Isotype
B53$_2$-Fc
PD-1
B53$_2$-Fc + PD-1
4-1BB
B53$_2$-Fc + 4-1BB
A
Mean %
CD3+
CD4+
Treg
CD8+
CD8/Treg ratio
B
Granulocytes
Macrophages
M1
M2
MDSCs
C
M1/M2 ratio
Monocytes
NK cells
NKT cells

Figure 21:

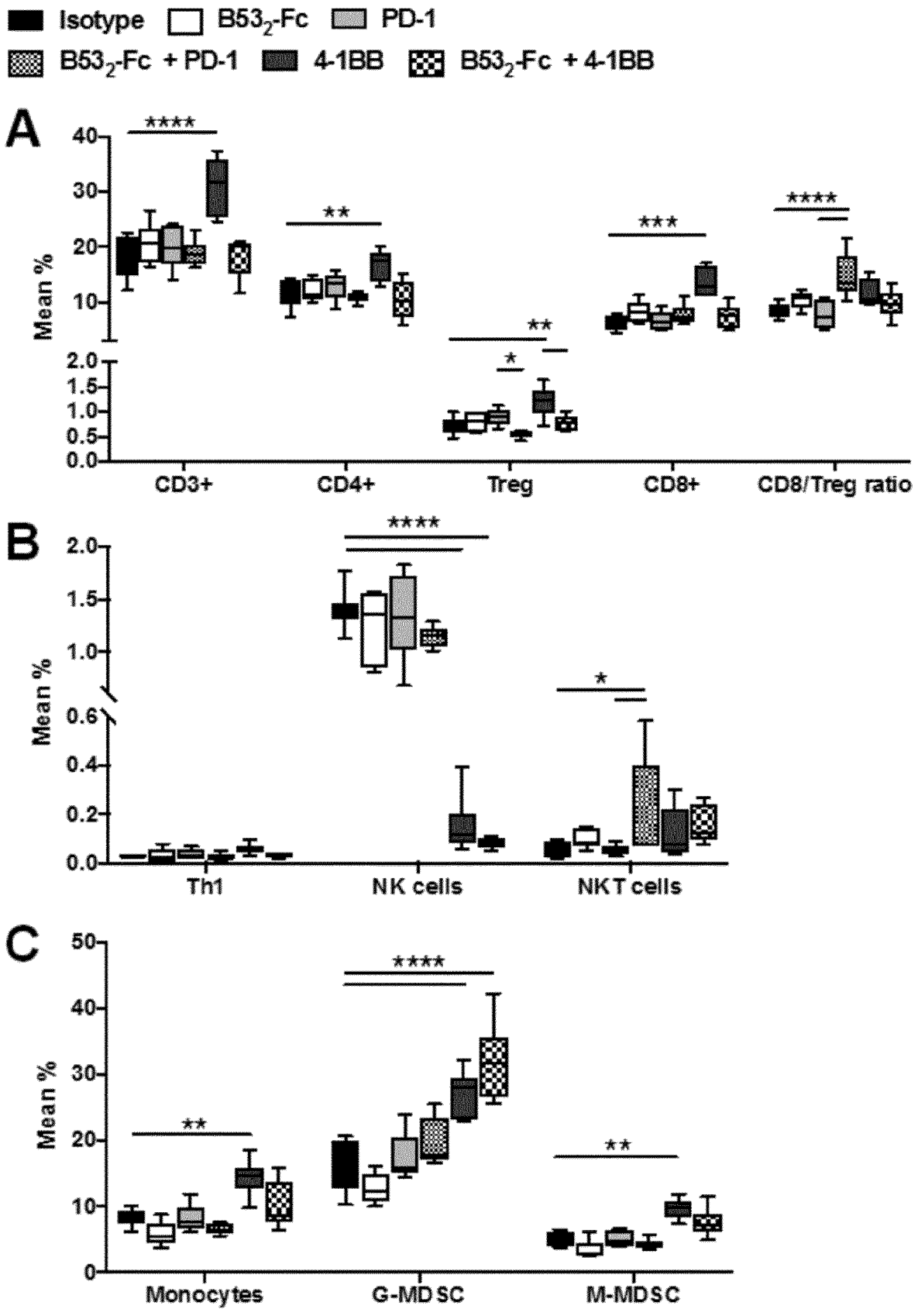

Isotype
B53$_2$-Fc
PD-1
B53$_2$-Fc + PD-1
4-1BB
B53$_2$-Fc + 4-1BB
A
Mean %
CD3+
CD4+
Treg
CD8+
CD8/Treg ratio
B
Th1
NK cells
NKT cells
C
Monocytes
G-MDSC
M-MDSC

Figure 22:

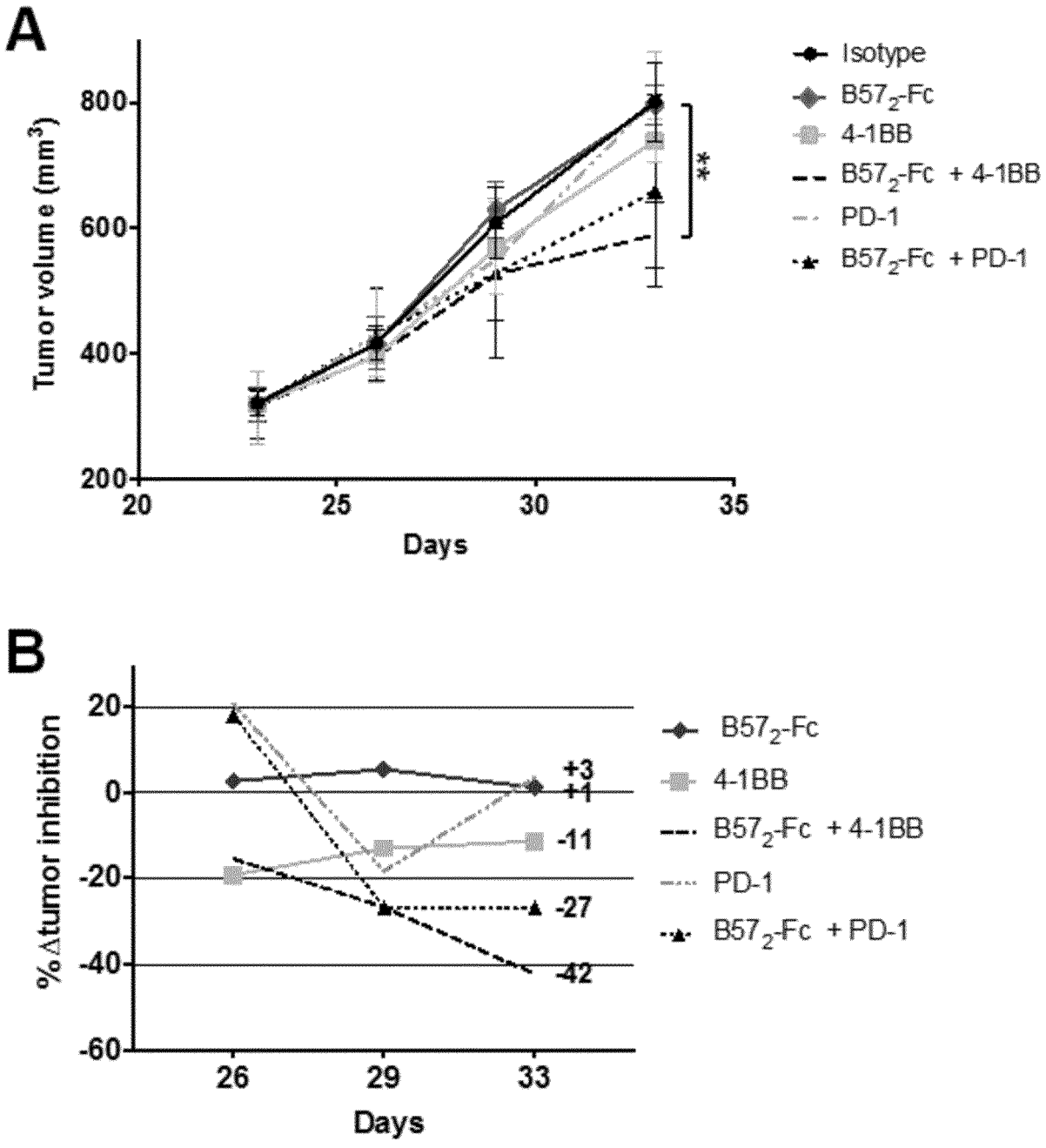

A
Tumor volume (mm3)
800
600
400
200
20
25
30
35
Days
**
Isotype
B57$_2$-Fc
4-1BB
B57$_2$-Fc + 4-1BB
PD-1
B57$_2$-Fc + PD-1
B
% Δtumor inhibition
20
0
-20
-40
-60
26
29
33
Days
+3
+1
-11
-27
-42
B57$_2$-Fc
4-1BB
B57$_2$-Fc + 4-1BB
PD-1
B57$_2$-Fc + PD-1

Figure 23:

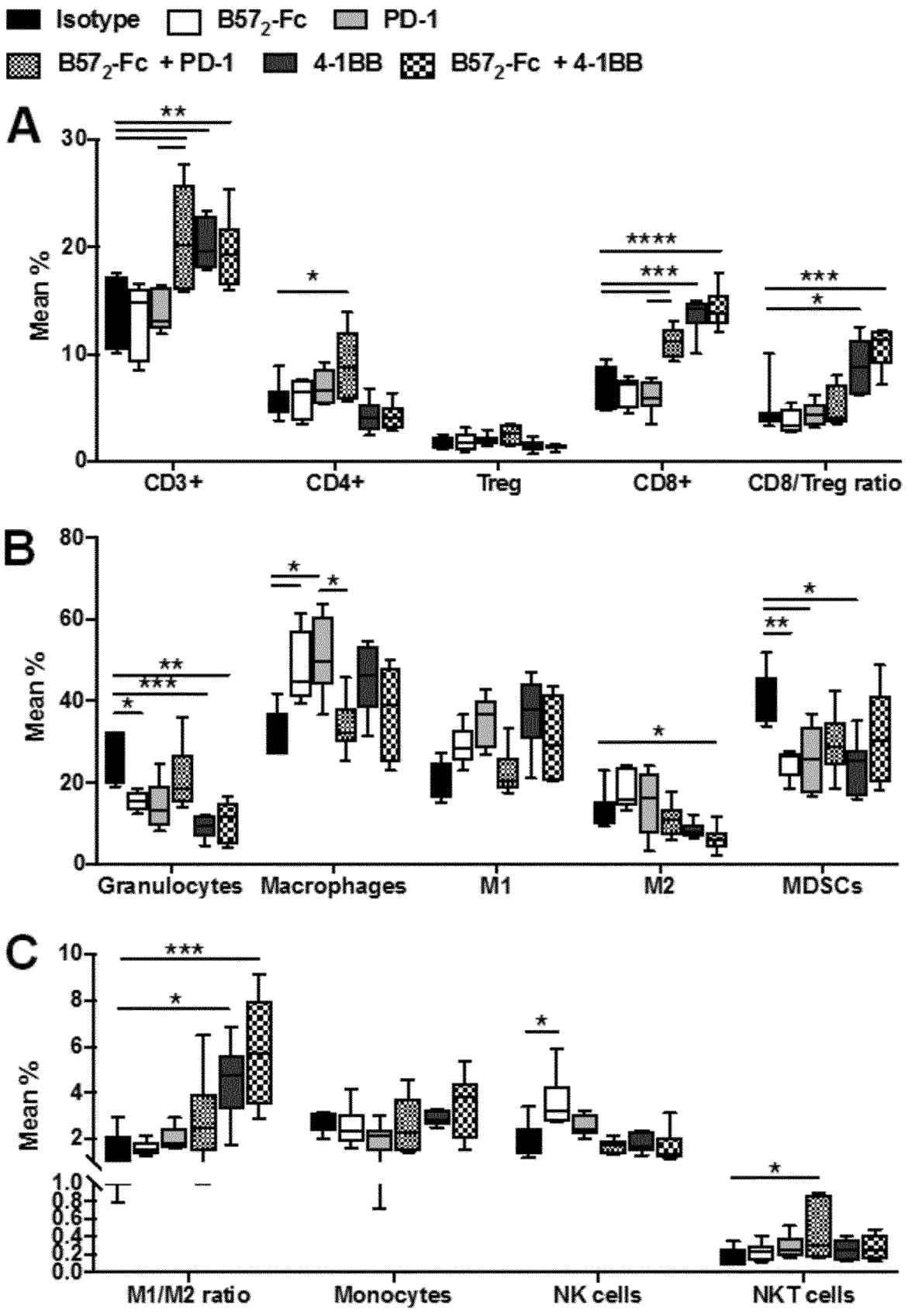

Isotype
B57$_2$-Fc
PD-1
B57$_2$-Fc + PD-1
4-1BB
B57$_2$-Fc + 4-1BB
A
Mean %
CD3+
CD4+
Treg
CD8+
CD8/Treg ratio
B
Granulocytes
Macrophages
M1
M2
MDSCs
C
M1/M2 ratio
Monocytes
NK cells
NKT cells

Figure 24:

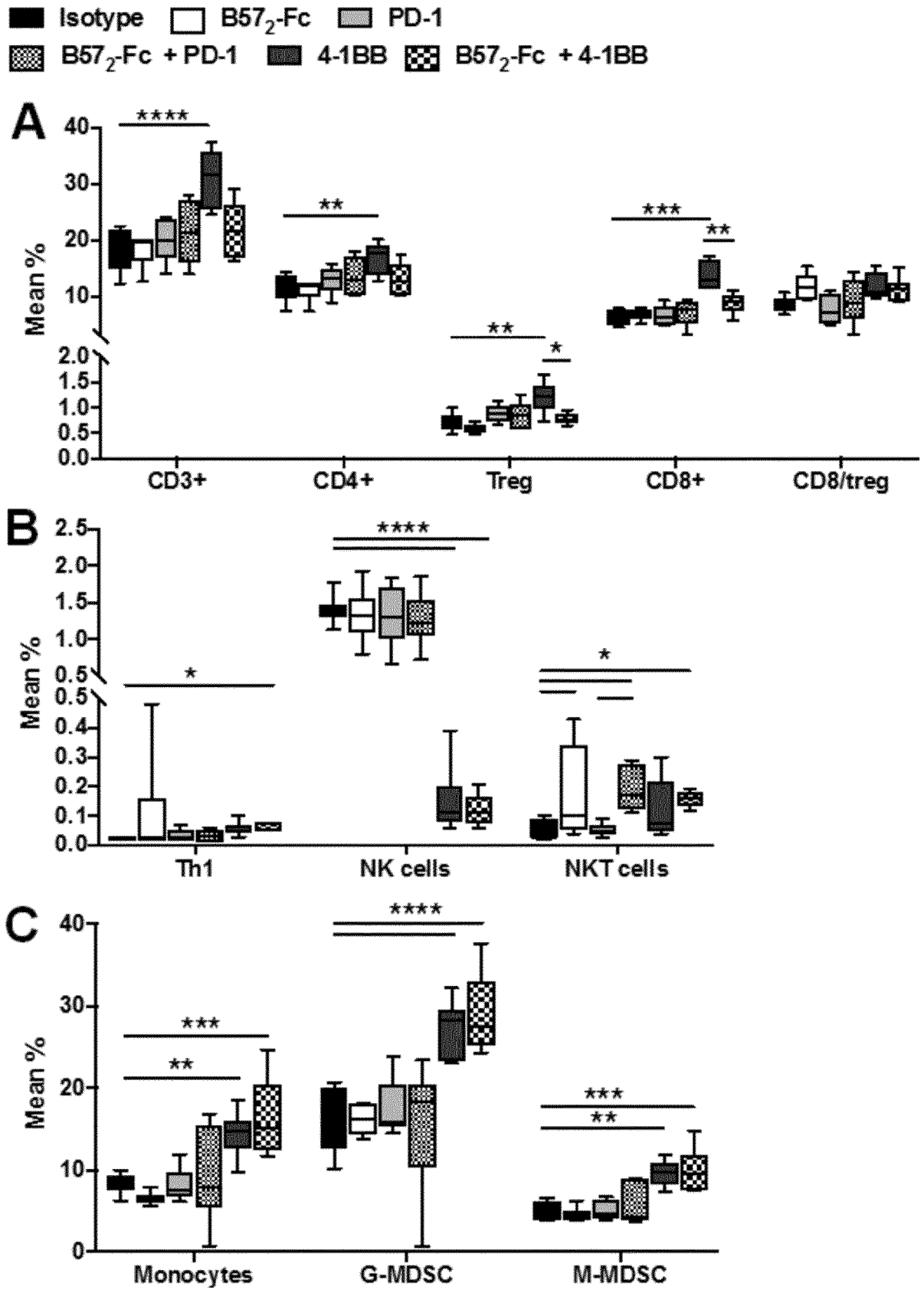

Isotype
B57$_2$-Fc
PD-1
B57$_2$-Fc + PD-1
4-1BB
B57$_2$-Fc + 4-1BB
A
Mean %
CD3+
CD4+
Treg
CD8+
CD8/treg
B
Th1
NK cells
NKT cells
C
Monocytes
G-MDSC
M-MDSC

Figure 25:

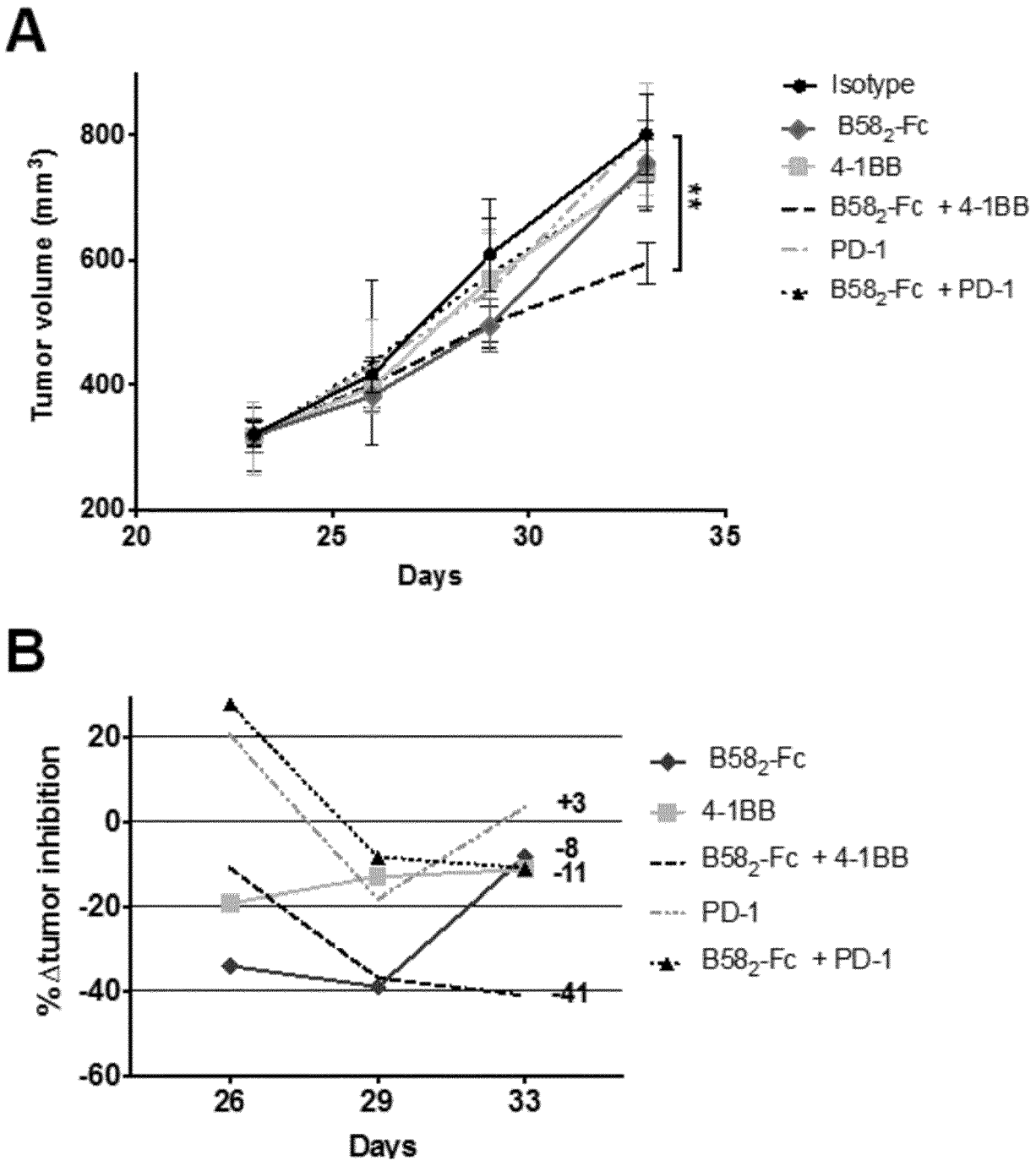

A
Tumor volume (mm3)
800
600
400
200
20
25
30
35
Days
**
Isotype
B582-Fc
4-1BB
B582-Fc + 4-1BB
PD-1
B582-Fc + PD-1
B
% Δtumor inhibition
20
0
-20
-40
-60
26
29
33
Days
+3
-8
-11
-41
B582-Fc
4-1BB
B582-Fc + 4-1BB
PD-1
B582-Fc + PD-1

Figure 26:

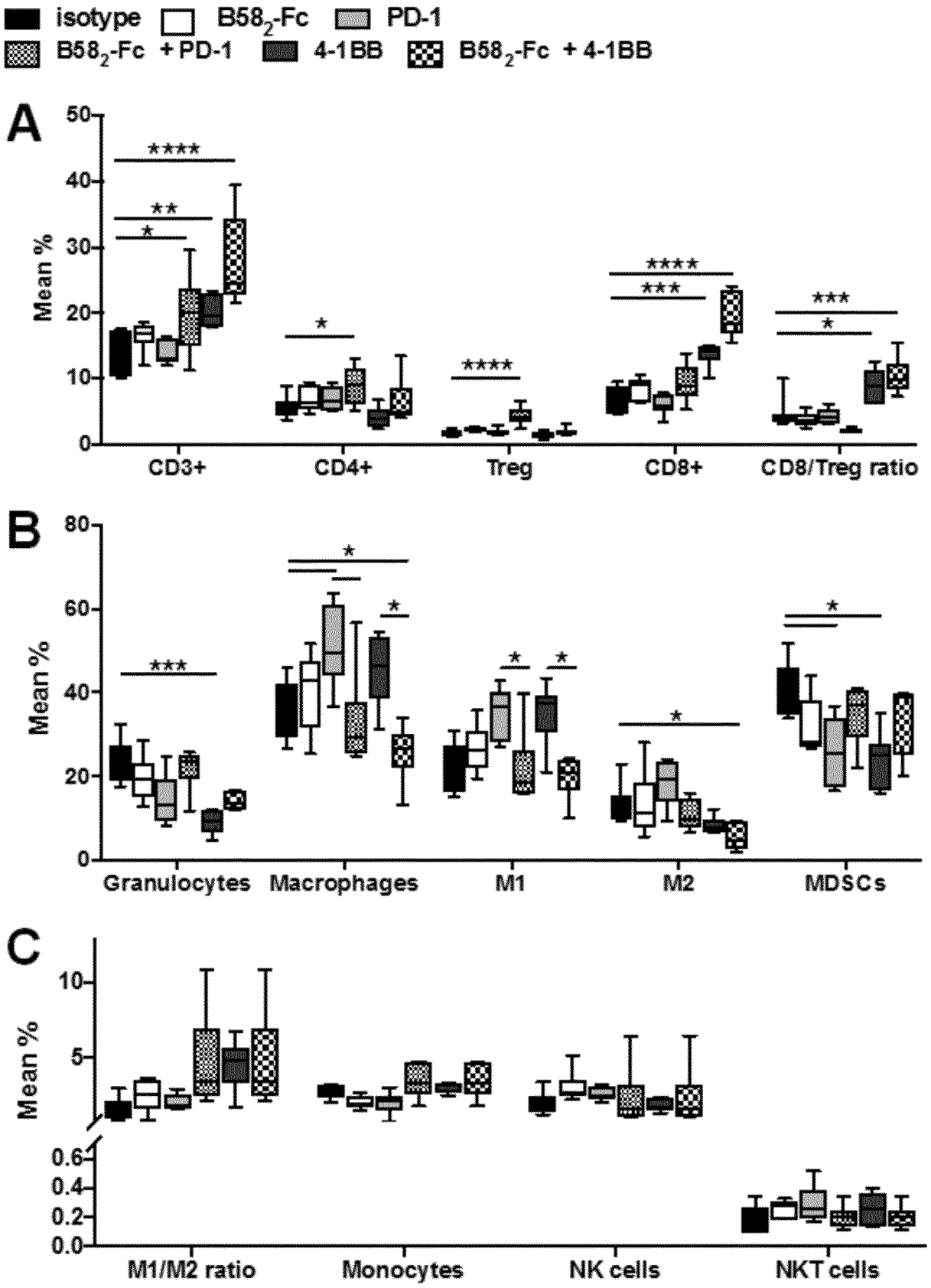

isotype
B58$_2$-Fc
PD-1
B58$_2$-Fc + PD-1
4-1BB
B58$_2$-Fc + 4-1BB
A
Mean %
CD3+
CD4+
Treg
CD8+
CD8/Treg ratio
B
Granulocytes
Macrophages
M1
M2
MDSCs
C
M1/M2 ratio
Monocytes
NK cells
NKT cells

Figure 27:

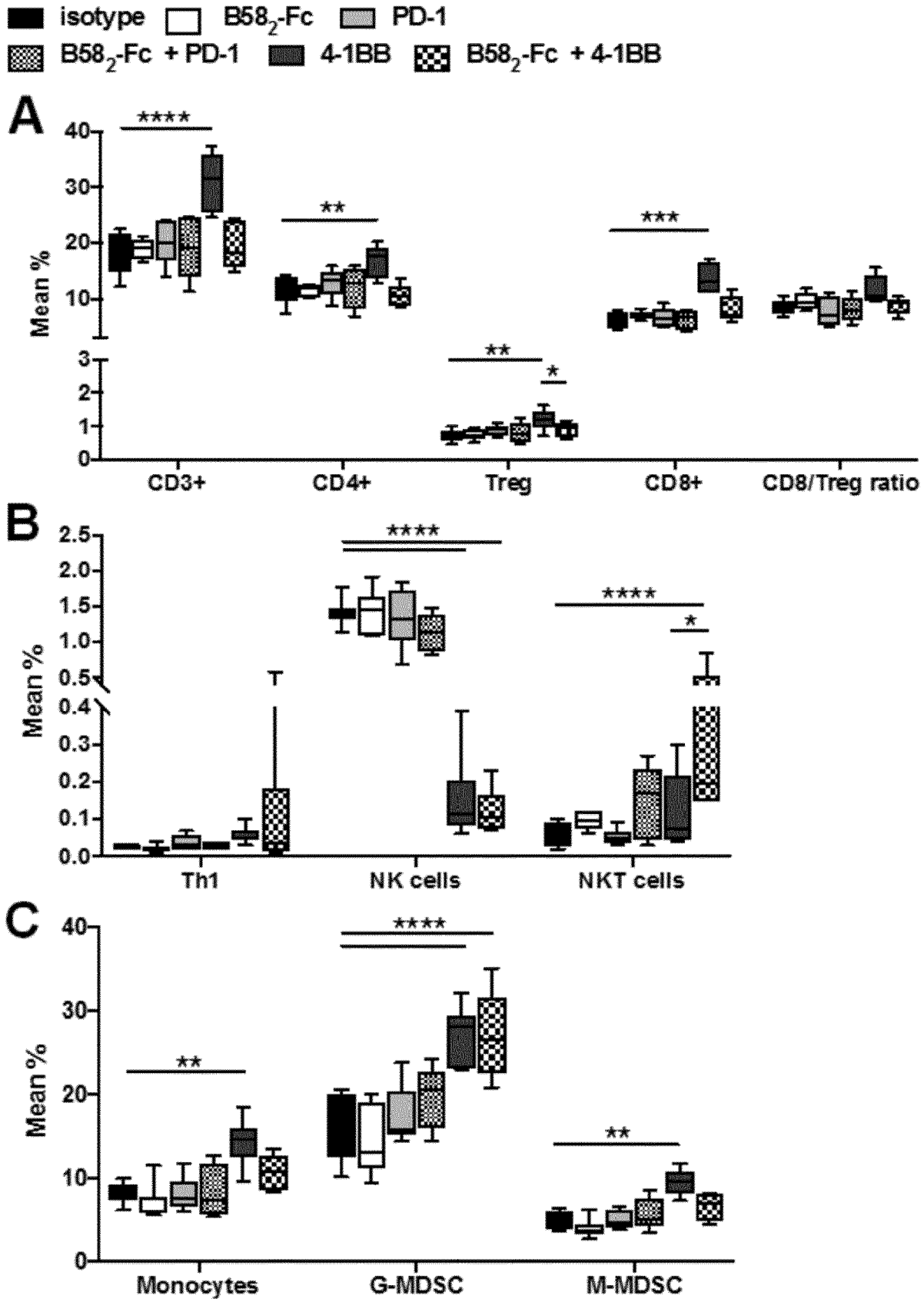

isotype
B58$_2$-Fc
PD-1
B58$_2$-Fc + PD-1
4-1BB
B58$_2$-Fc + 4-1BB
A
Mean %
CD3+
CD4+
Treg
CD8+
CD8/Treg ratio
B
Mean %
Th1
NK cells
NKT cells
C
Mean %
Monocytes
G-MDSC
M-MDSC

Figure 28:

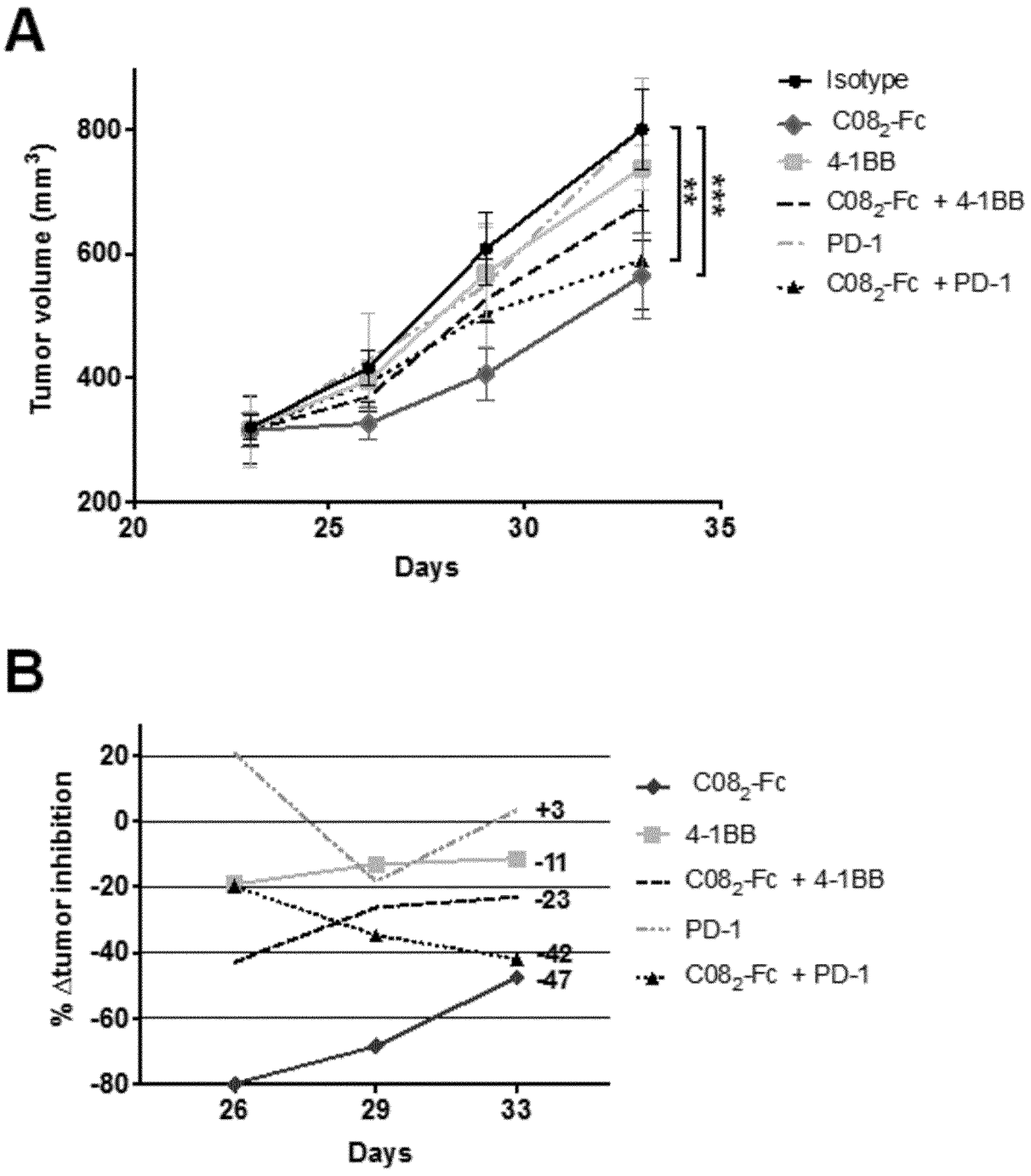

A
Tumor volume (mm3)
800
600
400
200
20
25
30
35
Days
**
***
Isotype
C08$_2$-Fc
4-1BB
C08$_2$-Fc + 4-1BB
PD-1
C08$_2$-Fc + PD-1
B
% Δtumor inhibition
20
0
-20
-40
-60
-80
26
29
33
Days
+3
-11
-23
-42
-47
C08$_2$-Fc
4-1BB
C08$_2$-Fc + 4-1BB
PD-1
C08$_2$-Fc + PD-1

Figure 29:

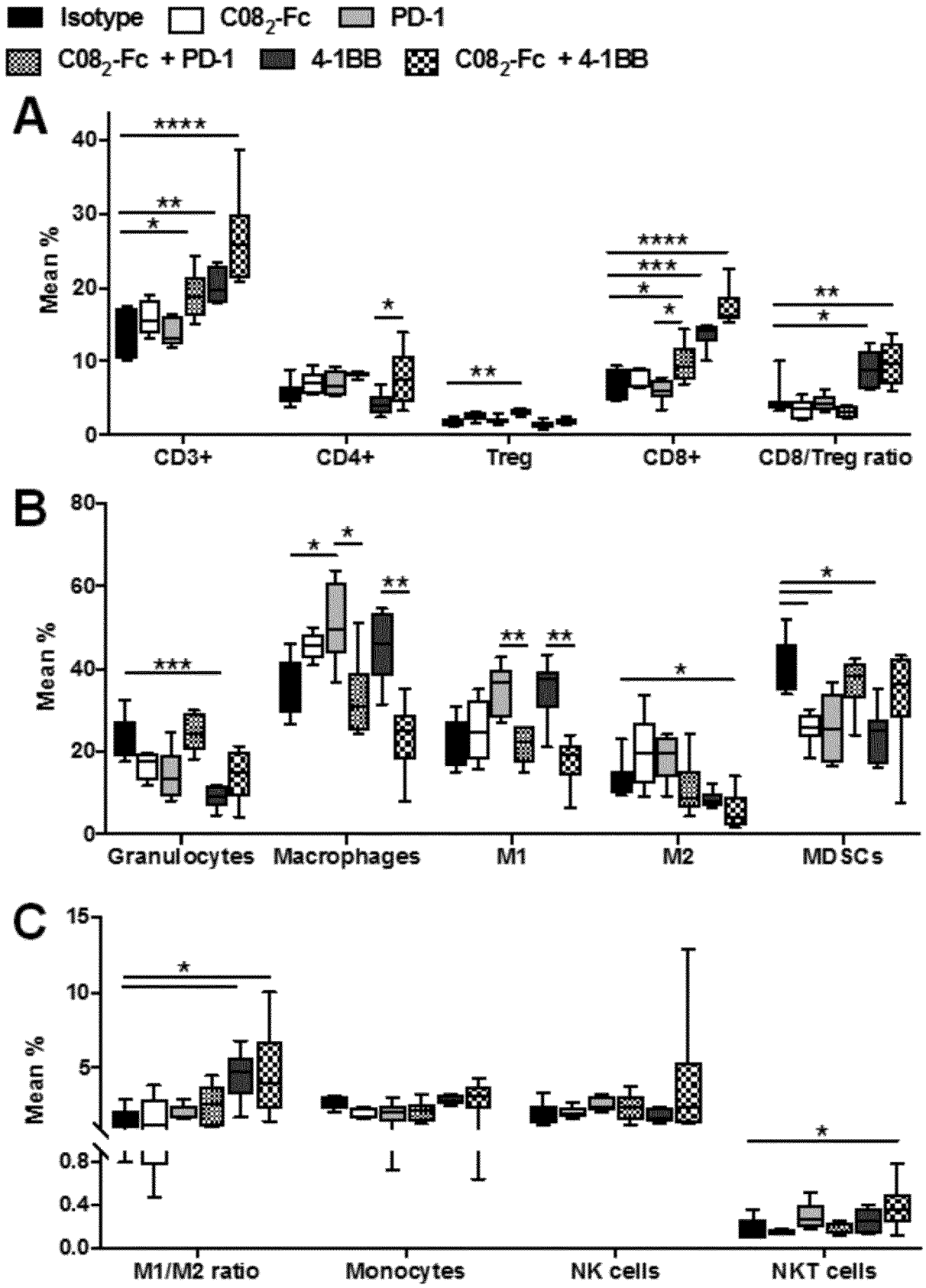

Isotype
C08$_2$-Fc
PD-1
C08$_2$-Fc + PD-1
4-1BB
C08$_2$-Fc + 4-1BB
A
Mean %
CD3+
CD4+
Treg
CD8+
CD8/Treg ratio
B
Granulocytes
Macrophages
M1
M2
MDSCs
C
M1/M2 ratio
Monocytes
NK cells
NKT cells

Figure 30:

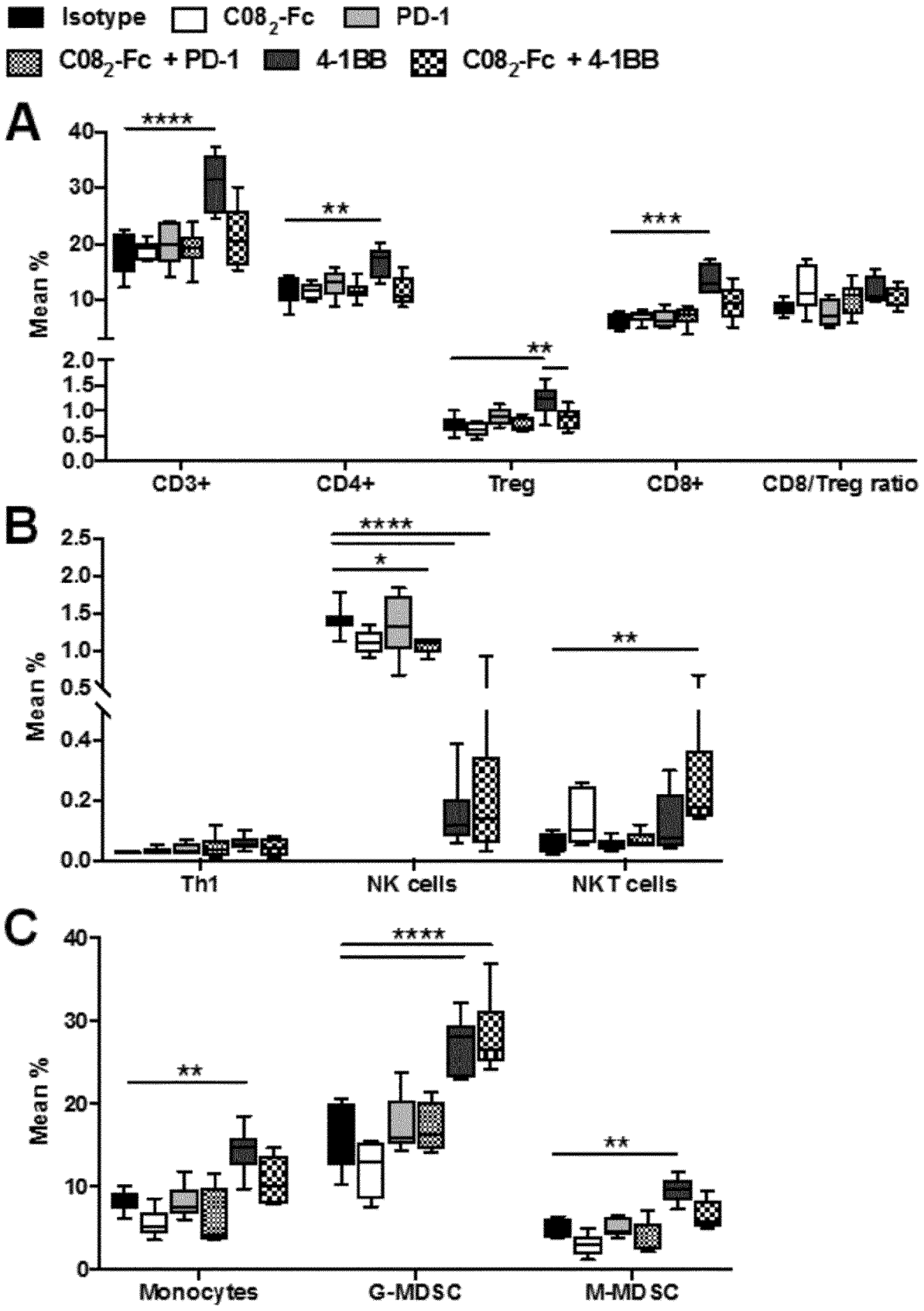

Isotype
C08$_2$-Fc
PD-1
C08$_2$-Fc + PD-1
4-1BB
C08$_2$-Fc + 4-1BB
A
Mean %
CD3+
CD4+
Treg
CD8+
CD8/Treg ratio
B
Th1
NK cells
NKT cells
C
Monocytes
G-MDSC
M-MDSC

Figure 31:

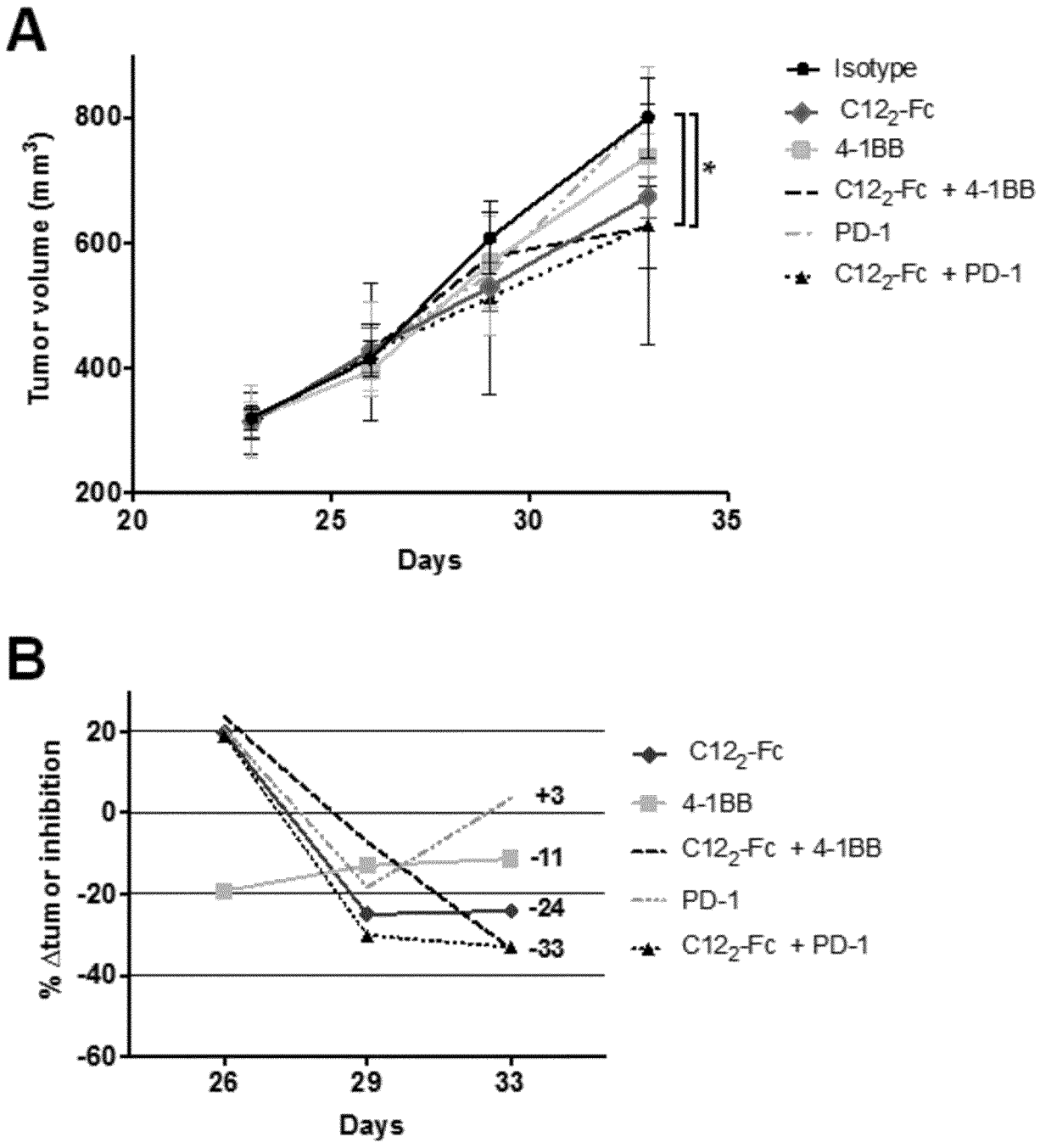

A
Tumor volume (mm3)
800
600
400
200
20
25
30
35
Days
*
Isotype
C12₂-Fc
4-1BB
C12₂-Fc + 4-1BB
PD-1
C12₂-Fc + PD-1
B
% Δtumor inhibition
20
0
-20
-40
-60
26
29
33
Days
+3
-11
-24
-33
C12₂-Fc
4-1BB
C12₂-Fc + 4-1BB
PD-1
C12₂-Fc + PD-1

Figure 32:

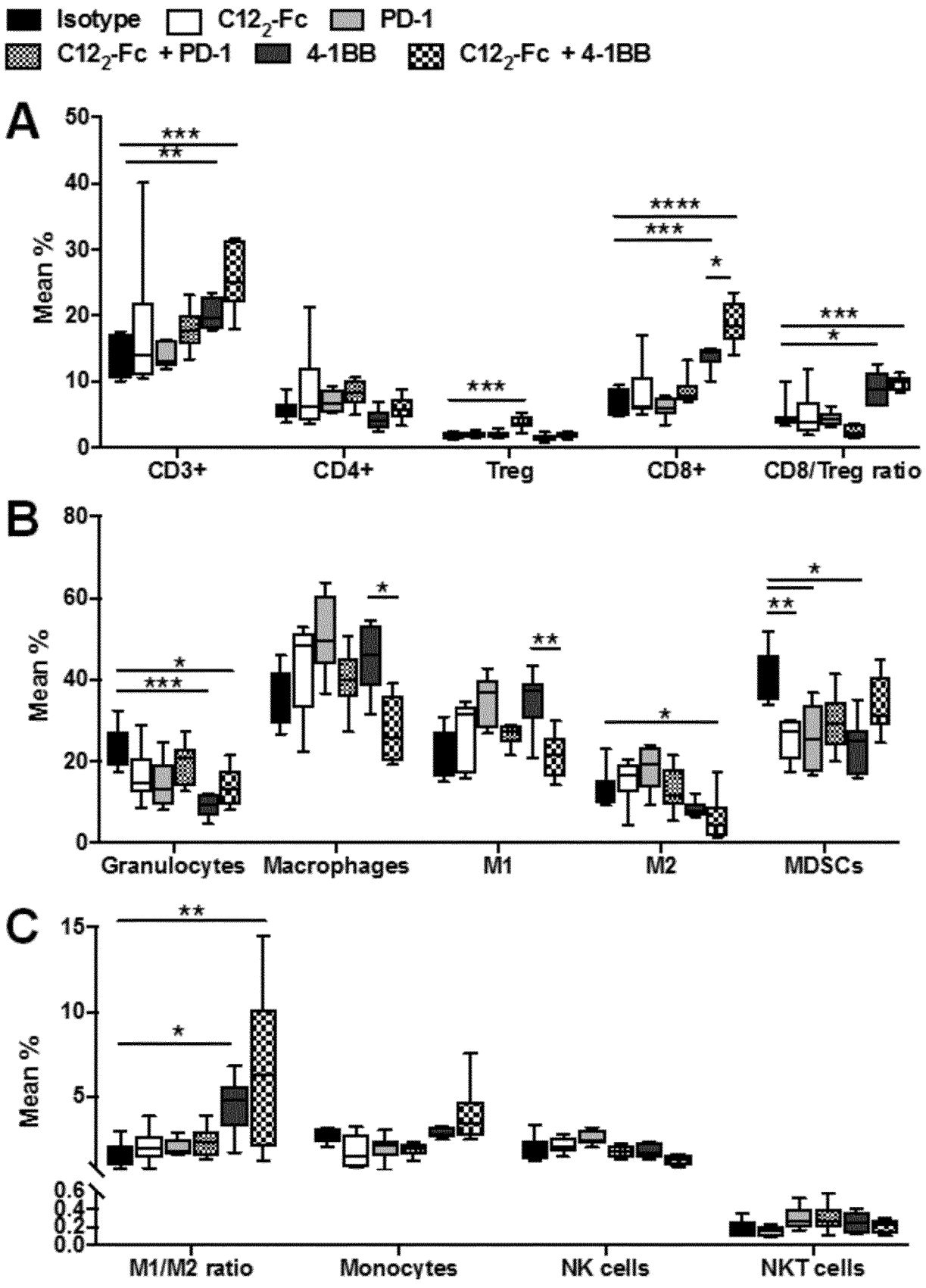
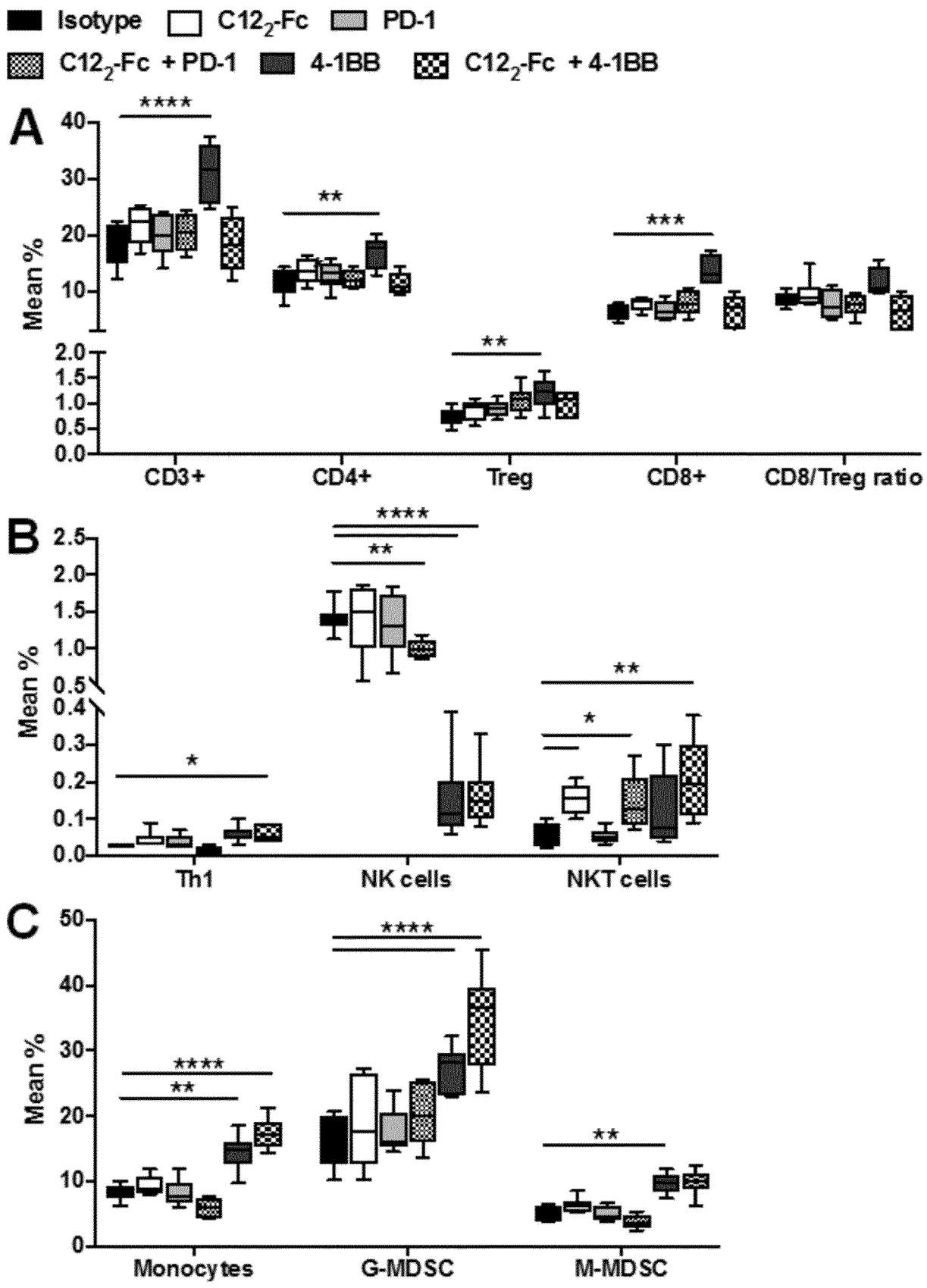

Isotype
C12$_2$-Fc
PD-1
C12$_2$-Fc + PD-1
4-1BB
C12$_2$-Fc + 4-1BB
A
Mean %
CD3+
CD4+
Treg
CD8+
CD8/Treg ratio
B
Granulocytes
Macrophages
M1
M2
MDSCs
C
M1/M2 ratio
Monocytes
NK cells
NKT cells

Figure 33:


Isotype
C12$_2$-Fc
PD-1
C12$_2$-Fc + PD-1
4-1BB
C12$_2$-Fc + 4-1BB
A
Mean %
CD3+
CD4+
Treg
CD8+
CD8/Treg ratio
B
Mean %
Th1
NK cells
NKT cells
C
Mean %
Monocytes
G-MDSC
M-MDSC

REFERENCES CITED IN THE DESCRIPTION

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